Interleukin-7 Induces the Proliferation of Normal Human B-Cell Precursors

By Sem Saeland, Valérie Duvert, Dominique Pandrau, Christophe Caux, Isabelle Durand, Nicholas Wrighton, Janus Wideman, Frank Lee, and Jacques Banchereau

In the present study, we investigated the effects of human recombinant interleukin-7 (IL-7) on the proliferation of enriched hematopoietic cells isolated from human adult and fetal bone marrow (BM), in cultures of CD34+ cells, IL-7 was found to induce dose-dependent incorporation of [3H]-thymidine ([3H]-Tdr), but had no demonstrable effect on the development of myeloid colony-forming cells. Numbers of B-cell precursors (BCP), initially present within CD34+ populations and which included a CD34+ CD20+ subset, were significantly increased when CD34+ BM cells were cultured in the presence of IL-7. This effect was most striking on CD20+ BCP, and resulted at least partly from higher numbers of cycling cells as indicated by Hoechst 33342 fluorescence (Calbiochem, Behring Diagnostics, La Jolla, CA). These results indicate that IL-7 promotes the growth of BCP within the CD34+ compartment. In line with the B-lineage affiliation of CD34+ target cells, committed BCP (CD10+ CD19+ surface IgM+) isolated from BM were also found to proliferate in response to IL-7. Interestingly, this effect of IL-7 was strongly potentiated by the addition of IL-3. Taken together, and in accordance with previous observations on murine cells, our data indicate that IL-7 acts as a growth factor during the ontogeny of human B lymphocytes.

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MATERIALS AND METHODS

Collection and isolation of human cells. Human BM samples were collected according to institutional guidelines. Adult marrow was obtained by liiac crest aspiration from donors free of hematologic disease through the courtesy of Prof P. Hervé (Besançon, France). Fetal femurs were obtained after abortions between 18 and 24 weeks of pregnancy, and were kindly provided by Prof J.L. Touraine (Lyon, France). Fetal marrow was collected by scraping and flushing the femurs, and subsequently filtering on nylon wool to remove bone fragments.

Light-density mononuclear cells were isolated by Ficoll-Hypaque (density = 1.077) (Eurobio, Paris, France) centrifugation. Mononuclear fractions were depleted of adherent cells as previously described. Cells bearing CD34+ antigen were subsequently isolated from nonadherent mononuclear fractions by immune "panning" with anti-My10 (HPCA-1; Becton-Dickinson, Mountain View, CA) anti-CD34 monoclonal antibody (MoAb), as reported in detail elsewhere. This procedure routinely yielded preparations consisting of greater than 98% CD34+ cells as judged by FACScan flow cytometric analysis with anti-My10 MoAb (see the next section for details). In some experiments, after overnight incubation at 37°C, the purified CD34+ cells were labeled with murine B1 anti-CD20 MoAb (Coulter, Hialeah, FL), followed by immunomagnetic beads (30 beads/cell) coated with antimouse Ig (Dynabeads M450; Dynal, Oslo, Norway). CD20+ cells were subsequently depleted from the CD34+ populations by use of a magnet.

Cells expressing CD10 antigen were isolated from mononuclear BM samples by immune panning with the ALB-1 anti-CD10 MoAb (Immunotech, Marseille, France), as described previously for purification of CD34+ cells. Subsequent purity of the CD10+ fractions was greater than 98% as evaluated by cytofluorimetric

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analysis after staining with anti-CD10 (see below). In experiments on fetal BM, surface (s) IgM+ cells were further removed from the CD10- fractions by immunomagnetic bead depletion following labeling with an anti-μ chain MoAb (Immunotech), as described previously. This step was necessary to obtain a population of precursor cells because CD10 is a pan-B cell antigen in fetal bone marrow.24

**Cytofluorimetric analysis.** Cells were stained for cell-surface antigen expression by standard immunofluorescence techniques using a panel of MoAbs. HPCA-1 (CD34) (Becton-Dickinson) and anti-human IgM (μ chain) (Immunotech) were shown to fluoresce isothiocyanate (FITC)-goat F(ab')2 anti-mouse Ig heavy chain (Biocon, Meudon, France). Other MoAbs used were directly conjugated and included CALLA-FITC (CD10), Leu 12-FITC (CD19), Leu 16-FITC (CD20) (all from Becton-Dickinson); J5-PE (phycoerythrin) (CD10), B1-FITC (CD20) (Coulter); IOM34-FITC (CD34), IOB4-PE (CD19), and IOB1a-FITC (CD21) (Immunotech). For all MoAbs, negative controls were provided by monoclonal mouse Ig of the same isotype but unrelated specificity. In the case of cytoplasmic μ chain (μc) analysis, cells were permeabilized with saponin (0.3% wt/vol) before and during labeling with anti-μ antibody and FITC-conjugate.

Double surface-membrane fluorescence was performed by simultaneous staining with FITC and PE-conjugated MoAbs.

Fluorescence was analyzed on a FACSScan flow cytometer (Becton-Dickinson, Sunnyvale, CA). Gating was set according to forward and wide-angle light-scatter parameters to exclude subcellular particles from acquisition data. Data were subsequently processed using the LYSYS program (Becton-Dickinson).

**Analysis of cellular DNA content was performed by labeling cells with the DNA-specific stain Hoechst 33342 (10 μmol/L) (Calbiochem, Behring Diagnostics, La Jolla, CA) after an 18-hour incubation (37°C) with 0.1 μg/mL colcemid (GIBCO, Grand Island, NY) to block cell division. Cells were further stained for expression of membrane antigens with FITC-conjugated MoAbs, before analysis on a FACSA 440 flow cytometer (Becton-Dickinson).

**Cell cultures.** Liquid cultures were performed at 37°C and 5% CO2 in RPMI 1640 medium supplemented with 10% vol/vol heat-inactivated fetal calf serum, 10 mmol/L HEPES, 2 mmol/L L-glutamine, 5 x 10-5 mol/L 2-mercaptoethanol, penicillin (100 U/mL), and streptomycin (100 μg/mL) (hereafter referred to as complete medium). For 3H-thymidine (3H-TdR) incorporation studies, cells were seeded at 104 cells/100 μL in round-bottom 96-well microtiter plates (Nunc, Roskilde, Denmark). Cells were pulsed with 1 μCi methyl-3H-TdR (CEA, Saclay, France; specific activity 25 Ci/mmol) during the last 6 hours of culture, and subsequently harvested. Results were expressed as mean counts per minute (cpm) ± standard deviation (SD) of triplicate wells. Cells were also seeded at 3 x 104 cells/mL complete medium in 24-well plates (Linbro; Flow Laboratories, McLean, VA) for subsequent cell counts, phenotyping, and cell-cycle analysis.

** Colony-forming unit assays were performed by plating 2 x 104 CD34+ cells/mL medium containing 0.9% (wt/vol) methylcellulose and supplemented with 1 IU recombinant human erythropoietin (Epo) (Amgen, Thousand Oaks, CA; specific activity >7 x 105 IU/mg), as previously described in detail.22** Duplicate dishes were plated in each experiment, and after 14 days of incubation at 37°C and 5% CO2, colonies (>50 cells) were counted using phase-contrast microscopy. The clonogenic capacity of CD10- sIgM+ cells was also evaluated by plating 104 cells/mL CFU medium (without Epo). After 14 days of incubation, duplicate plates were monitored for the presence of clusters (10 to 20 cells).

**Cytokines.** Human IL-7 coding sequences were cloned using polymerase chain reaction (PCR) on total library plasmid DNA derived from the EBV-positive Burkitt lymphoma line Raji (kindly supplied by Dr K. Yokota, DNAX, Palo Alto, CA). Conditions for PCR were those recommended by Perkin Elmer Cetus (Norwalk, CT) and reactions on 1 μg of library plasmid DNA were performed in a thermal cycler from this supplier. Primers used were: sense strand, 5'CTACCGCGCGCTCCCGACAGCATG (362 through 387); antisense strand, 5'GGTGCGTTTGTATTGCCCACCTC (950 through 926) (coordinates taken from the Immunex human IL-7 cDNA sequence). In addition, to facilitate direct and unidirectional cloning into a eukaryotic expression vector, a PstI site was incorporated into the 5'-end of the sense primer and a KpnI site into the 5'-end of the antisense primer. The PCR product was gel purified, cleaved with PstI and KpnI, and inserted between the PstI and KpnI sites of the pCDKs296 expression vector.23 The insert was then sequenced and shown to be identical to that reported by Goodwin et al.24 Cos7 cells were transfected with this plasmid and serum-free supernatants were collected. Human IL-7 was purified from Cos7 supernatant using a two-step Sephacryl S-100 (Pharmacia, Uppsala, Sweden) chromatography followed by hydroxylapatite high-performance liquid chromatography. This procedure yielded a preparation of IL-7 which titered 5 x 106 units/mL as measured by proliferation of clone-K cells, a mouse pre-B-cell line responsive to human IL-7.25 One unit of activity was defined as the amount of IL-7 inducing half-maximal proliferation of clone-K cells. Unless otherwise specified, IL-7 was used at 20 U/mL. Several of the experiments reported in the present study have been repeated using Escherichia coli-derived human IL-7 purified to homogeneity (2.5 x 104 U/mg on clone K cells), obtained through R & D Systems (Minneapolis, MN). No significant differences were observed in the dose-response when using comparable numbers of IL-7 units from either Cos7 or E coli-derived preparations, indicating that the biologic effects of the Cos7 preparation are indeed due to IL-7. Purified yeast-expressed human recombinant IL-3 was kindly provided by Dr S. Tindall (Schering-Plough Research, Bloomfield, NJ). Specific activity was of 5 x 105 U/mg, 1 U corresponding to half-maximal 3H-TdR incorporation by human CD34+ cells. In the present study, IL-3 was used at a concentration of 10 ng/mL, previously shown to be saturating for myeloid development of CD34+ cells.26

**RESULTS**

IL-7 induces dose-dependent 3H-TdR incorporation in liquid cultures of CD34+ cells, but lacks myeloid colony-stimulating activity. We investigated the capacity of IL-7 to induce the proliferation of adult and fetal CD34+ cells. First, 104 purified BM CD34+ cells were seeded in suspension culture in the presence of various concentrations of IL-7. As shown in Fig 1, IL-7 was able to enhance 3H-TdR uptake by CD34+ cells in a dose-dependent manner, with maximum effects reached at 12 U/mL IL-7. The peak of the IL-7 response was seen between days 3 and 7 of culture (not shown). The results presented in Table 1 show that IL-7 was able to enhance 3H-TdR uptake by CD34+ cells originating from both adult and fetal BM. However, the magnitude of IL-7-dependent proliferation (2.58 ± 0.43; mean stimulation index ± SEM, n = 6) was always lower than that observed in the presence of IL-3, which activates a high proportion (25% to 40%) of CD34+ cells to proliferate.26 Interestingly, IL-7 failed to induce significant 3H-TdR incorporation by CD34+ cells isolated from cord blood (five experiments, data not shown).

BM CD34+ cells were then seeded in semisolid medium in the presence of Epo, under optimal conditions, for the
Fig 1. Concentration-dependent increase in ^H-TdR incorporation by fetal BM CD34+ cells, after culture for 6 days in the presence of IL-7. Results are expressed as mean cpm ± SD of triplicate wells seeded with 10^6 CD34+ cells.

development of myeloid colony-forming cells. As illustrated in Table 2, IL-7 (20 U/mL) was found to lack detectable colony-stimulating activity. Furthermore, the addition of IL-7 did not modify the development of myeloid colonies observed in response to IL-3 (Table 2). In these assays, IL-7 was tested at concentrations ranging from 2.5 to 50 U/mL (data not shown). These results indicate that IL-7 does not appear to play a primordial role in early myelopoietic events of CD34+ cells.

Characterization of B-lineage cells within CD34+ populations. Taking into consideration that IL-7 is a growth factor for murine BCP, we speculated that the IL-7-dependent proliferation observed above could reflect growth of human BCP contained within the heterogeneous CD34+ populations. To address this issue, we first characterized the B-lineage compartment within freshly isolated CD34+ cells. Double-color fluorescence indicated that adult BM CD34+ cells contained a relatively important BCP subset (28% of CD34+ cells in a representative experiment shown in Fig 2), as defined by coexpression of CD19 and CD10 antigens. While some CD34+ cells in adult BM expressed CD10 but lacked B-lineage CD19 antigen (Fig 2), CD10+ CD19- cells were not observed in fetal BM (see Fig 7). When compared with adult CD34+ cells, fetal CD34+ populations contained a higher proportion of BCP, which frequently constituted more than 50% of total CD34+ cells (Table 3).

In screening adult and fetal CD34+ populations for expression of other B-lineage markers, we observed that a subset of cells expressed CD20 antigen, as detected using either Leu-16 or B1 anti-CD20 MoAbs. Double-color fluorescence analysis (Fig 3) indicated that the CD20+ cells were indeed part of the CD34+ population (8.1% of the cells in the experiment presented) and did not represent contaminant CD34- cells. Accordingly, CD19+ CD10- cells corresponding to a mature B-cell phenotype were not detected among CD34+ cells (Fig 2). Immunomagnetic depletion of CD20+ cells from the CD34+ compartment resulted in a concomitant decrease in the percentage of CD10+ and CD19+ cells (Fig 4), illustrating that the CD34+ CD20+ cells indeed represent B-lineage cells. However, the CD34+ BCP compartment did not contain pre-B cells, as indicated by lack of cμ chain expression (not shown). Furthermore, we did not observe slgM+ cells (Fig 4) or CD21+ cells (not shown).

Table 2. IL-7 Lacks Myeloid Colony-Stimulating Activity on BM CD34+ Cells

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Adult Marrow</th>
<th>Fetal Marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>IL-7</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>IL-3</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>IL-7 + IL-3</td>
<td>106</td>
<td>116</td>
</tr>
</tbody>
</table>

Figures indicate numbers of colonies (> 50 cells) counted in duplicate 1-mL methylcellulose cultures, and correspond to values of representative experiments on adult and fetal CD34+ cells. IL-7 was used at 20 U/mL and IL-3 at an optimal colony-stimulating concentration (10 ng/mL). Epo (1 IU/mL) was added at initiation of the cultures. No significant differences were noted in colony-type distribution in the presence of IL-7 + IL-3, as compared with IL-3 alone.

Table 1. IL-7 Enhances 3H-TdR Incorporation in Liquid Cultures of BM CD34+ Cells

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>cpm x 10^3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medium</td>
</tr>
<tr>
<td>Adult marrow</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Fetal marrow</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
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<td></td>
<td>6</td>
</tr>
</tbody>
</table>

Figures represent mean cpm ± standard deviation of triplicate microcultures of purified CD34+ cells (10^5 cells/well) pulsed with 1 μCi of 3H-TdR on day 6 of culture.

*cpm IL-7/cpm medium.
Finally, we consistently failed to observe a BCP population among CD34+ cells isolated from cord blood (not shown).

**IL-7 increases the numbers of BCP in cultures of CD34+ cells.** We next designed experiments to determine whether the addition of IL-7 to CD34+ BM cultures would affect the number of B-lineage cells initially present. As illustrated in Table 3, IL-7 (20 U/mL) permitted the recovery, after 6 days, of a higher number of viable cells than control cultures lacking IL-7 (68.5% ± 8.1%, mean increase ± SEM, n = 6). Cells recovered after culture with IL-7 represented 70% to 140% of the input CD34+ cells (mean ± SEM = 111.6% ± 11.4%, n = 6). When compared with control cultures lacking IL-7, phenotypic analysis showed an average 380% and 970% respective increase in the numbers of CD10+ and CD20+ cells recovered in cultures in the presence of IL-7 (Table 3). When compared with input cells, IL-7 increased by an average of 82.4% the number of viable CD10+ cells and by 420% the number of viable CD20+ cells (Table 3). Increased recovery of CD19+ cells, as monitored in some experiments, further ascertained the B-lineage affiliation of cells affected by IL-7 (see experiment 1 of Table 3, and Fig 6).

Double-color fluorescence performed after 6 days of culture of adult CD34+ cells in the presence of IL-7 indicated that all CD20+ cells were CD10+ (Fig 5). Furthermore, the CD20+ cells expressed CD19, but failed to display cμ, sIgM, or CD21, even when analyzed after 10 to 12 days.

| Table 3. IL-7 Increases the Numbers of CD10+ and CD20+ Cells in Liquid Cultures of CD34+ BM Cells |
|----------------------------------------------------------|--------------------------|--------------------------|
| Experiment No.  | Day 0  | Day 0  | Day 6  | Day 6  | Day 6  | Day 6  |
|                | Total  | CD10+  | CD20+  | CD10+  | CD20+  | CD10+  | CD20+  |
| Adult marrow   | 1,000  | 205    | 44     | 542    | 106    | 27     | 1,092  | 493    | 239    |
|                | 2,000  | 282    | 42     | 459    | 131    | 10     | 705    | 344    | 95     |
|                | 1,000  | 284    | 32     | 518    | 103    | 29     | 906    | 277    | 130    |
|                | 1,000  | 182    | 56     | 723    | 95     | 21     | 1,176  | 393    | 298    |
| Fetal marrow   | 1,000  | 316    | 78     | 983    | 115    | 19     | 1,430  | 843    | 269    |
|                | 1,000  | 536    | 69     | 792    | 307    | 32     | 1,387  | 947    | 309    |
| Mean ± SEM     | 1,000  | 301 ± 51| 53 ± 7 | 669 ± 82| 143 ± 33| 23 ± 3 | 1,116 ± 114| 549 ± 114| 223 ± 37 |

*Number of CD10+ and CD20+ cells were calculated as (total viable cells x percentage labeled cells obtained by fluorescence-activated cell sorter (FACScan) analysis x 10^-6). Day 6 figures represent recovery from 10^6 input CD34+ cells. In experiment 1, numbers of CD19+ cells were also monitored and, in the presence of IL-7, were found to be specifically increased 3.1-fold as compared with input cells.*
Fig 3. Double-fluorescence analysis of CD34 and CD20 expression on CD34+ cells isolated from adult BM. Negative controls were provided by FITC and PE-conjugated MoAbs of unrelated specificity.

Fig 4. Expression of B-lineage-associated antigens on CD34+ cells isolated from adult BM before (left panel) and after (right panel) depletion of CD20+ cells, illustrating coexpression of CD20, CD10, and CD19 antigens, and lack of slgM+ cells. Numbers represent percentage positive cells after subtracting background staining (not shown) obtained with an unrelated MoAb.
of culture (data not shown). Taken together, these data indicate that IL-7 induces an expansion of the precursor B-cell compartment in liquid cultures of BM CD34+ cells.

In contrast, addition of IL-7 to CD34+ cells from cord blood had no significant effect on the number of cells recovered in culture (not shown). In this respect, we observed no detectable BCP in cord blood cultures despite the presence of IL-7. 

**IL-7 induces the proliferation of BCP.** We next investigated whether the observed IL-7–dependent expansion of BCP from the CD34+ compartment reflected proliferation of committed precursors. Several approaches were taken to address this issue.

First, cord blood CD34+ cells, which do not contain BCP as indicated by the lack of CD10 and CD19 markers, did not proliferate in response to IL-7, and failed to express B-lineage markers after culture in the presence of IL-7 (not shown).

Second, we performed simultaneous analysis of DNA content (Hoechst 33342 incorporation) and surface phenotype of cells recovered after short-term exposure of adult CD34+ cells to a saturating concentration of IL-7 (20 U/mL). In line with data presented previously, CD34+ cells cultured for 96 hours (including a final 18-hour colcemid pulse) contained higher proportions of CD10+, CD19+, and CD20+ cells in the presence of IL-7, as compared with medium alone (Fig 6). Notably, an important fraction (50% to 70%) of the B-lineage cells (CD19+ and CD20+) found in the IL-7 cultures was in the S and G2/M phases of cycle, as indicated by high Hoechst 33342 fluorescence (Fig 6). Furthermore, whereas significant spontaneous proliferation of CD34+ cells was observed in medium alone, addition of IL-7 did not result in an increase of cycling CD10+, CD19+, or CD20+ cells (Fig 6). These data indicate that IL-7 preferentially permits the proliferation of BCP in cultures of CD34+ cells, which accounts at least in part for the expansion of B-lineage cells observed previously.

Third, CD10+ sIgM- cells isolated from fetal BM, which represent committed BCP expressing CD19 antigen (Fig 7), also proliferated in response to IL-7 (Table 4). Finally, we identified IL-7–dependent clonogenic cells within the CD10+ sIgM- population (Table 5). These clonogenic cells were found at a frequency between 1/500 and 1/3000, as visualized by the appearance of clusters comprised of 10 to 20 cells. The detection of clonogenic cells in cultures seeded at relatively low density (10⁴ cells/mL) supports the notion
that the proliferative effect of IL-7 is directly exerted on BCP.

IL-7–induced proliferation of BCPs is potentiated by IL-3. As IL-3 has been described to act as a growth factor in murine and human B-cell ontogeny, we tested the effects of this cytokine, in combination with IL-7, on the proliferative response of committed BCP.

Data illustrated in Table 4 show that IL-3 was constantly less active than IL-7 in inducing the proliferation of CD10+ slgM− BCP. However, the combination of IL-3 and IL-7, at saturating concentrations, resulted in levels of \(^{3}H\)-TdR uptake that were higher than the sum of \(^{3}H\)-TdR uptake induced by each factor alone. Potentiating effects of IL-7 and IL-3 were further confirmed by viable cell counts performed on day 7 of culture (Table 4).

Phenotypic analysis showed that the majority of CD10+ slgM− cells cultured for 7 days in the combined presence of IL-7 and IL-3 remained CD10+. Furthermore, all cells recovered in the cultures displayed CD19, thus showing their B-lineage affiliation (Fig 7). Interestingly, CD19 and CD20 antigens were hyperexpressed on cells cultured in IL-7 and IL-3 when compared with freshly isolated CD10+ cells. The majority of the cells cultured in the presence of IL-7 and IL-3 were still BCP as judged by lack of slgM expression at day 7 (76.4% slgM− cells in the experiment presented in Fig 6). Finally, slgM+ cells emerged in the IL-7 + IL-3 cultures (Fig 7), but a comparable number of slgM+ cells were also found in cultures performed in medium alone (not shown), showing "spontaneous" appearance of slgM as described earlier. These results indicate that IL-7 and IL-3 do not affect the maturation step to slgM+ B cells and subsequent proliferation of slgM+ cells.
Fig 7. Phenotypic analysis of CD10<sup>-</sup> slgM<sup>-</sup> cells isolated from fetal BM, as determined on day 0 (upper panel fluorescence histograms) and after 7 days of culture (lower panel) in the combined presence of IL-7 (20 U/mL) and IL-3 (10 ng/mL).

DISCUSSION

The present study was undertaken to evaluate the effects of IL-7 on the proliferation of enriched human hematopoietic cells, with particular reference to the role of this cytokine in B-cell ontogeny.

Herein we showed that IL-7 did not stimulate the growth of myeloid CD34<sup>+</sup> progenitors, in line with reports indicating that IL-7 does not affect murine myelopoiesis. In contrast, we showed that IL-7 permitted an expansion of the BCP compartment in cultures of CD34<sup>+</sup> cells. Because CD34 antigen is only present on the most immature BCP, our data show that IL-7 acts early in human B-cell development. This effect of IL-7 represented a proliferation of B-lineage cells, most of which expressed CD20 antigen. We found that CD20<sup>+</sup> cells were initially present within freshly isolated CD34<sup>+</sup> populations, contrasting with the notion that loss of CD34 antigen precedes expression of CD20 in B-cell ontogeny. In this respect, the scarcity of CD34<sup>+</sup> CD20<sup>+</sup> cells (approximately 0.1% of mononuclear BM cells) likely explains why this subset was not detected in earlier studies. Our observed expansion of CD20<sup>+</sup> BCP in the presence of IL-7 strongly suggests that the CD34<sup>+</sup> CD20<sup>+</sup> subset is a preferential target for the effects of IL-7.

The finding that IL-7 promotes the proliferation of purified CD10<sup>-</sup> slgM<sup>-</sup> cells expressing CD19 antigen confirmed that IL-7 acts on committed B-lineage precursors, in line with the reported presence of IL-7 receptors on normal human BCP. In addition, we identified IL-7-dependent clonogenic cells within the CD10<sup>-</sup> slgM<sup>-</sup> fetal populations. The clonogenic cells develop into clusters in semisolid medium when CD10<sup>-</sup> slgM<sup>-</sup> cells are plated at low density, which supports the notion that the proliferative effects are directly exerted by IL-7 and not indirectly mediated through possible contaminant accessory cells.

We are currently exploring the range of IL-7 target cells within CD10<sup>-</sup> slgM<sup>-</sup> populations, in particular to deter-
IL-7 AND HUMAN B LYMPHOPOIESIS

Table 5. Clonalogenic Growth of CD10⁺ sIgM⁻ Cells in the Presence of IL-7

<table>
<thead>
<tr>
<th>Clusters/10⁶ Cells</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>2, 1</td>
<td>0, 2</td>
</tr>
<tr>
<td>IL-7 (20 U/mL)</td>
<td>25, 15</td>
<td>32, 37</td>
</tr>
</tbody>
</table>

Fetal CD10⁺ sIgM⁻ cells were seeded at 10⁵/mL methylcellulose medium, as described in Materials and Methods.

*Figures correspond to numbers of day 14 clusters (10 to 20 cells) identified in duplicate plates (1 mL) in two separate experiments.

mine whether responsive cells all express CD34 antigen, which was borne by an average 20.1% of fetal CD10⁺ sIgM⁻ cells (mean of seven experiments, data not shown).

We failed to show any effect of IL-7 on CD34⁺ cells lacking a BCP subset (isolated from cord blood), as determined both by absence of enhanced proliferation and lack of induction of CD10, CD19, or CD20 antigens (not shown). These data suggest that IL-7 does not act through a recruitment of early uncommitted cells toward the B-lineage pathway.

We observed that the proliferation detected on BM populations in response to IL-7 was always of a relatively modest magnitude, as measured both by [3H]-TdR uptake and increase in cell numbers. These results suggested that, within the populations analyzed, IL-7 target cells were present at low frequency and/or did not have high proliferative capacity. Consistent with a low frequency of CD34⁺ target cells, cell-cycle analysis showed that IL-7 preferentially permits the proliferation of a subset of cells within the BCP compartment. In addition to a low frequency of target cells, our results indicate that the proliferative capacity of responding cells appears to be relatively low, as shown by an average 4.2-fold expansion of the CD20⁺ BCP compartment in 6-day cultures of CD34⁺ cells. Because the majority of the CD20⁺ cells were shown to respond to IL-7, this may only represent 2 to 3 cycles per cell. In contrast, murine studies have shown that IL-7 induces the development of B-lineage colonies ranging up to 1,000 cells, which implies 10 cycles, assuming that all cells divide. Also, as monitored by [3H]-TdR uptake, the effects of IL-7 appear of a much higher magnitude in the murine system. This does not appear to represent a bias due to our utilization of CD34⁺ cells, which include a majority of myeloid cells, because we observed similar relatively modest effects of IL-7 on total committed B-precursor populations (CD10⁺ sIgM⁻). The lower magnitude of the proliferative effects of IL-7 on human BCP as compared with murine BCP may reflect a physiologic difference between these species. However, a more likely possibility may be that optimal culture conditions for human BCP remain to be established. We found important differences in BCP numbers in cultures of CD34⁺ cells performed with and without IL-7, in apparent discordance with a low magnitude of [3H]-TdR uptake. These results suggest that IL-7 maintains viability of most BCP, but yet is only able to sustain limited growth under our experimental conditions.

In this context, we further showed that the combination of IL-7 and IL-3 potentiated the growth of CD10⁺ sIgM⁻ cells, indicating that optimal BCP development is likely to require an association of cytokines. We are presently investigating whether IL-7 and IL-3 act on the same cells, or on different target populations.

We did not observe the appearance of cμ⁺ pre-B cells during culture of CD34⁺ BM cells in IL-7 (data not shown), even as CD10⁺ CD20⁺ cells represented approximately 20% of total cells. This finding indicates that IL-7-responsive cells in such cultures are upstream of the pre-B stage. These target cells may correspond to the IL-7-responsive murine B220⁺ cμ⁻ pro-B cells obtained from WW cultures. However, in the mouse B-lineage IL-7-responsive cells in fresh BM have been reported to be largely B220⁺ cμ⁻ pre-B (cμ⁺) cells. A complete analysis of the comparative range of IL-7-responsive BCP in the human and mouse awaits further studies on the effects of IL-7 on CD34⁺ BCP, which include the human pre-B (cμ⁺) cells. In this respect, lack of cμ expression and markers of mature B cells in cultures of CD34⁺ cells in the presence of IL-7 would be compatible with murine data reporting that IL-7 does not induce BCP maturation.

Finally, we were unable to maintain cells beyond 2 weeks of culture despite the presence of IL-7, thus indicating that the present culture conditions are not yet optimal for long-term growth of human BCP. In keeping with this, we are currently investigating growth and maturation signals required by early BCP in association with IL-7, with particular emphasis on the respective contribution of soluble cytokines and physical association with BM stromal cells.

In conclusion, in addition to being a growth factor for normal human T cells and malignant T- and B-lymphoid precursors, our data show that IL-7 acts as a growth factor in early normal human B-cell ontogeny.

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