The FLK2/FLT3 Ligand Synergizes With Interleukin-7 in Promoting Stromal-Cell-Independent Expansion and Differentiation of Human Fetal Pro-B Cells In Vitro

By Reiko Namikawa, Marcus O. Muench, Jan E. de Vries, and Maria-Grazia Roncarolo

The effects of a novel cytokine FLK2/FLT3 ligand (FL) on human fetal bone marrow-derived CD34+CD19+ pro-B cells were analyzed in a stromal-cell-independent, serum-deprived culture system. FL, like interleukin-3 (IL-3), synergized with IL-7 in promoting pro-B cell growth, and differentiation of these cells into CD34+CD19+ IgM+ slgM- pre-B cells, whereas a small proportion of these cells even differentiated into more mature slgM+ B cells. In contrast, KIT ligand (KL) and granulocyte-macrophage colony-stimulating factor (GM-CSF) were ineffective in promoting IL-7-dependent pro-B cell growth and differentiation. Maximal levels of pro-B cell expansion, generally resulting in 15- to 30-fold increases in cellularity, were obtained in cultures supplemented with optimal doses of FL + IL-7 + IL-3. The addition of mouse bone marrow stromal cells further enhanced the proliferation and differentiation of pro-B cells obtained in the presence of these three cytokines. Under these conditions, cultures could be maintained for more than 4 weeks, and in general 40- to 50-fold increases in cell numbers were observed by 3 weeks of culture. The percentages of IgM+ and slgM+ B cells increased 1.5- to 3-fold and 2-fold, respectively, suggesting that stromal cells may provide additional costimulatory signals for human B-cell growth and differentiation that are different from IL-7, IL-3, and FL. Collectively, our results indicate that FL, in contrast to KL, strongly promotes long-term expansion and differentiation of human pro-B cells in the presence of IL-7 or in combination of IL-7 and IL-3, which is a novel property of this hematopoietic growth factor.

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kines and stromal cells in regulating early human B-cell development.

MATERIALS AND METHODS

Monoclonal antibodies (MoAbs). Biotinylated, phycoerythrin (PE)-labeled, or fluorescein isothiocyanate (FITC)-labeled control antibodies or MoAbs against the following antigens were purchased from Becton Dickinson (San Jose, CA) or an otherwise stated vendor: CD3 (SK7), CD8 (SK1), CD14 (MOP9), CD16 (GO22), CD19-PE (4G7) (Catag Laboratories, South San Francisco, CA), CD33-PE (PE67.7), CD34-FITC (BG12), CD34-biotin (ICH-3) (Catag Laboratories), CD56 (MY31), glycoporphin A (GPA) (10F7MN, obtained from American Type Tissue Culture Collection, Rockville, MD), and human IgM-FITC (Nordic Immunological Laboratories, Tilburg, The Netherlands). Mouse IgG1-PE and mouse IgG1-FITC (Catag Laboratories) were used as control antibodies. Allophycocyanin (APC)-conjugated streptavidin was purchased from Becton Dickinson.

Cytokines. Recombinant human (rh) IL-7, rhKL, and rhIL-3 were purchased from R&D Systems, Inc (Minneapolis, MN). rhGM-CSF was provided by Schering-Plough Corp (Kenilworth, NJ). Recombinant murine (rm) FL produced at our institute was used in this study because rmFL and rhFL were shown to have similar activities in stimulating cells expressing human FLK2/FLT3 receptor.26 Purification of FL was described previously in detail.24 Unless otherwise stated, KL, GM-CSF, and IL-3 were used at 20 ng/ml and FL was used at 50 or 100 BaF(2)T cells.

Isolation of human fetal BM progenitors. Human fetal limb bone marrow was obtained from Advanced Bioscience Resources (Alameda, CA) and was used with the approval of the Committee for the Protection of Human Subjects at our institute. The gestational ages ranged from 18 to 24 weeks. A single tissue was used in each experiment.

Fetal BM cells were subjected to density centrifugation using a 1.085 Accudenz gradient (Accurate Chemical & Scientific Corp, Westbury, NY) for 25 minutes at 1,000 g. The light-density BM cells were washed and resuspended in staining buffer consisting of phosphate-buffered saline (PBS) supplemented with 1 mmol/L EDTA, 50 µg/mL gentamicin, 0.5% human γ-globulins (Sigma Chemical Co, St Louis, MO), and 2% normal mouse serum. Throughout all staining procedures and cell sorting, cells were kept at 4°C. Before labeling the cells with MoAbs, the cells were preincubated in staining buffer for 10 minutes followed by staining with MoAb for 30 minutes in the same solution. To further enrich B-cell progenitors, the light-density BM cells were depleted of GPA+ or GPA-CD3+CD8+CD14+CD16+CD56+ cells using immunomagnetic beads (Dynal Inc, Great Neck, NY) as previously described.20 The depleted BM cells were then stained with MoAbs against CD19 (PE) and CD34 (FITC) or CD34 (biotin), followed by staining with streptavidin-APC, for the isolation of CD34-CD19+ and CD34+CD19+ cells using immunomagnetic beads (Dynal Inc, Great Neck, NY) as previously described.20 The depleted BM cells were then stained with MoAbs against CD19 (PE) and CD34 (FITC) or CD34 (biotin), followed by staining with streptavidin-APC, for the isolation of CD34-CD19+ and CD34+CD19+ cells using immunomagnetic beads (Dynal Inc, Great Neck, NY) as previously described.20 The depleted BM cells were then stained with MoAbs against CD19 (PE) and CD34 (FITC) or CD34 (biotin), followed by staining with streptavidin-APC, for the isolation of CD34-CD19+ and CD34+CD19+ cells using immunomagnetic beads (Dynal Inc, Great Neck, NY) as previously described.20

RESULTS

CD34+CD19+ pro-B cells proliferate in response to cytokines. CD34+CD19+ pro-B cells and CD34+ CD19+ B cells were sorted from density gradient separated fetal BM cells, depleted of GPA+, CD3+, CD8+, CD14+, and CD56+ cells (Fig 1A). The sorted cells always exhibited greater than 96% purity on reanalysis of each population (not shown). Proliferation of these cells in the presence of different cytokines was examined by 3H-TdR incorporation after 10 days of culture in serum-deprived culture medium (Fig 2A). Under these conditions, IL-7 alone induced significant proliferation of CD34+CD19+ pro-B cells, whereas IL-3, GM-CSF, KL, and FL each were ineffective. However, IL-3 or FL
acted synergistically with IL-7, whereas no synergy was observed with GM-CSF or KL. The effects of IL-3 or FL on IL-7-induced proliferation of pro-B cells were dose-dependent and reached plateau levels at doses of 10 ng/mL of IL-3 or 50 U/mL of FL in combination with an optimal concentration of 20 ng/mL IL-7 (data not shown). The highest proliferative responses of the CD34+CD19+ pro-B cells were always observed when all three cytokines were added simultaneously at optimal concentrations (Fig 2B). In contrast to the pro-B cells, CD34+CD19+ BM-derived B cells failed to proliferate in response to IL-7 under these culture conditions. In addition, IL-3, FL, KL, or GM-CSF added in combination with IL-7 were ineffective (data not shown). These results indicate that the proliferative responses obtained in the cultures of CD34+CD19+ cells were not caused by contaminations by mature B cells.

**Long-term proliferation of CD34+CD19+ pro-B cells.** Because IL-3 and FL had synergistic effects on IL-7-induced pro-B cell proliferation as measured in 3H-TdR incorporation assays, we examined whether combinations of these cytokines could support long-term proliferation of pro-B cells in our serum-deprived medium. The expansion of pro-B cells cultured in the presence of various cytokines or cytokine combinations was analyzed after 10 to 26 days of culture. B-cell yields in cultures supplemented with IL-7 alone were low and an average increase in cellularity of only 2.5-fold was observed. However, 15- to 30-fold increases in cellularity were obtained in cultures supplemented with optimal doses of IL-7 + IL-3 + FL after 3 weeks of culture (Fig 3A). Consistent with the results obtained in the 3H-TdR incorporation assays, combinations of IL-7 and IL-3, or IL-7 and FL induced intermediate levels of pro-B cell expansion, whereas no cells were recovered from the cultures without cytokines (data not shown). These results indicate that under the present culture conditions combinations of IL-7, IL-3, and FL can drive human pro-B cell expansion in a stromal-independent fashion for as long as 4 weeks.

**Mouse BM stromal cells further enhance cytokine-driven human pro-B cell proliferation.** Murine BM fibroblastic stromal cells can support human B lymphopoiesis. Therefore, we investigated whether the capacity of combinations of IL-7, IL-3, and FL to induce pro-B cell proliferation and to drive long-term pro-B cell expansion could be further enhanced by the cloned mouse stromal cell line 30R, which is known to support mouse B lymphopoiesis in Whitlock-Witte cultures. The effect of 30R on pro-B cell proliferation was analyzed by comparing the results obtained from 3H-TdR incorporation assays performed in the presence or absence of irradiated stromal cells (Fig 2A and C). Coculture of pro-B cells with stromal cells alone or with stromal cells in the presence of FL, KL, IL-3, or GM-CSF did not result in significant 3H-TdR incorporation, despite the fact that 30R produces low amounts of IL-7 that are clearly not sufficient to induce human pro-B cell proliferation (D. Rennick, personal communication, 1994). However, addition of IL-7 to the cultures with stromal cells resulted in a pro-B cell proliferation that was in general threefold to sixfold higher than that observed with IL-7 alone. Furthermore, even in the presence of 30R, both FL and IL-3 had synergistic effects on IL-7-induced pro-B cell proliferation. Collectively, these results suggest that 30R expresses species cross-reactive costimulatory molecules, other than FL and KL, which potentiate IL-7-induced human pro-B cell proliferation.

Next, we investigated the effects of 30R on long-term human pro-B cell expansion. In cultures with stromal cells, but no exogenously added cytokines, pro-B cell viability was maintained and cell numbers were very modestly increased by a maximum of twofold. IL-7 alone induced a 15- to 30-fold expansion of pro-B cells in the presence of 30R (Fig 3B), which was considerably higher than the average 2.5-fold expansion obtained in the absence of 30R (Fig 3A). Addition of 30R to the pro-B cell cultures supplemented with optimal doses of IL-7 + IL-3 + FL promoted pro-B cell expansion even further. The increases in cellularity in cultures supplemented with IL-7 + IL-3 + FL were at all the time points consistently higher than those performed in the presence of IL-7 alone (Fig 3B). Although high standard errors were observed because of the donor variations, 1.63 ±
0.24-fold higher average cell yields were obtained in cultures with IL-7 + IL-3 + FL than in those supplemented with IL-7 alone (n = 10 tissues). In general, a 40- to 50-fold increase in cell numbers was obtained under this condition by the third week of culture. Pro-B cells of some donors showed extremely high degrees of proliferation under these culture conditions. With one donor an 80-fold increase was obtained at day 18, whereas pro-B cells of another donor could even be expanded 174-fold at day 14 (results not shown). Pro-B cell cultures initiated with IL-7 + IL-3, or IL-7 + FL in the presence of stromal cells yielded again cell numbers that were intermediate to those obtained in cell cultures stimulated with IL-7 alone and with the combination of all three cytokines (not shown). These results indicate that, although combinations of IL-7, IL-3, and FL induce a quite dramatic long-term expansion of human pro-B cells in serum-deprived medium, their proliferation was even further enhanced in the presence of stromal cells.

**Differentiation of CD34⁺CD19⁺ pro-B cells in culture.** Flow cytometric studies of cultured pro-B cells showed that most cells obtained from cultures initiated with CD34⁺CD19⁺ pro-B cells were CD19⁺ B cells (>95% of gated viable cells). Cells cultured in the presence of cytokinas had a high forward-scatter profile, irrespective of the presence or absence of stromal cells. In contrast, pro-B cells cultured with stromal cells, in the absence of exogenously added cytokinas, remained small in cell size (data not shown). Pro-B cells cultured in medium alone could not be analyzed because of the paucity of viable cells recovered.

Since the expression of Ig gene products are reliable markers for early human B-cell differentiation,⁴⁰,⁴¹ cytoplasmic and surface IgM expression by pro-B cells cultured in the presence of cytokinas was analyzed (Fig 4). In accordance with a previous report,⁴⁰,⁴¹ it was found that 5% to 10% of freshly isolated CD34⁺CD19⁺ cells expressed detectable amounts of clgM, whereas most of CD34⁺CD19⁺ cells were clgM⁺ (Fig 1B and C). However, no slgM⁺ cells could be detected in the former population (not shown). In Table 1, it is shown that the percentage of clgM⁺ cells significantly increased in stroma-free cultures to 22-46%. In addition, 0.2% to 6.6% of the cells expressed slgM, irrespective of whether they were cultured in the presence of IL-7 alone or IL-7 in combination with IL-3, FL, or IL-3 + FL. These results indicate that IL-7 supports partial differentiation of pro-B cells, whereas FL and IL-3, which both enhanced IL-7-induced pro-B cell proliferation, do not enhance B-cell differentiation under these culture conditions. In contrast, higher percentages of clgM- and slgM-expressing cells were obtained in the B cells cultured with 30R compared to stroma-free cultures, indicating that stromal cells further enhanced IL-7-driven human pro-B cell differentiation. Again, addition of...
IL-3 or FL to these cultures did not induce significant changes in the percentages of cIgM+ or sIgM+ cells.

In addition to acquiring IgM expression, pro-B cells lose CD34 expression during the B-cell differentiation process. In Table 2 it is shown that in the absence of stromal cells, both the percentages and absolute numbers of CD34+CD19+ cells were reduced after 10 to 21 days of culture. In addition, no significant differences in the percentages and absolute numbers of CD34+CD19+ cells could be detected in cultures supplemented with IL-7 or with IL-7 in combination with IL-3 or FL, or IL-3 + FL. These data further support the notion that IL-7 induces pro-B cells to differentiate into CD34- cIgM+ or sIgM+ B cells under the present culture conditions. However, it is of interest to note that pro-B cell cultures performed in the presence of stromal cells always contained percentages of CD34+ cells that were considerably higher than those cultured in the absence of stromal cells (Fig 4). These CD34+CD19+ cells could be maintained in cultures for as long as 33 days, which was the latest time period analyzed. Determination of the absolute numbers even showed that the CD34+CD19+ cells increased 1.5- to 3.2-fold in stromal-cell-containing cultures. In contrast, in the absence of 30R, CD34+CD19+ cells represented, in general, less than 20% of the initial cell number cultured with IL-7 + IL-3 + FL.

Collectively, these data indicate that although stromal cells, as judged by the induction of cIgM and sIgM expression, further enhanced B-cell differentiation induced by IL-7, they also simultaneously support the maintenance and expansion of the CD34+CD19+ population in vitro, whereas combinations of IL-7 + IL-3 + FL in the absence of stromal cells failed to do so.

**DISCUSSION**

In the present study, it is shown that highly purified human fetal BM-derived CD34+CD19+ pro-B cells can be induced by cytokines to proliferate and to differentiate in serum-deprived medium, in a stromal-cell-independent manner. In the presence of combinations of IL-7, IL-3, and FL the pro-B cells continued to proliferate for as long as 4 weeks, resulting in up to 30-fold increases in cellularity by the third week of culture. Furthermore, the percentage of cIgM+ pre-B cells were significantly enhanced, whereas a small proportion of the cells even differentiated into sIgM+ B cells under these culture conditions. B-cell differentiation as judged by the acquisition of cIgM and sIgM expression in these cultures correlated with a loss of CD34 expression on these cells. Previous studies have shown that fetal BM-derived CD10+ sIgM- cells could only be induced to proliferate in the presence of human BM fibroblastic stromal cells and IL-7. More defined CD34+CD10+ cIgM- pro-B cells were shown to proliferate in the presence of mouse fibroblasts (L cells) and IL-7, or IL-7 + IL-3. These CD10+CD34+ cIgM- pro-B cells, but not CD10+CD34- cIgM+ pre-B cells, were reported to proliferate upon IL-7 stimulation under serum-deprived conditions in a stromal-cell-dependent manner. However, the cell expansion and its duration observed in the present stroma-free cultures are considerably greater than those observed in previously described, stromal-cell-dependent human B-cell progenitor culture systems. The choice of using a serum-deprived culture medium was based on previous studies demonstrating the importance of selected batches of FCS in supporting long-term mouse B-cell cultures, suggesting the presence of serum-derived growth modulators. The use of serum-deprived culture conditions was also critical to determine the effect of cytokines acting as synergistic factors, such as FL. It has to be noted that our serum-deprived medium was superior to FCS containing RPMI-1640-based Whitlock-Witte culture medium in driving pro-B cell proliferation (data not shown), suggesting that yet to be defined medium components affect the growth of human pro-B cells.

IL-3 and FL, which failed to drive pro-B cell proliferation on their own, had costimulatory activity on IL-7-induced pro-B cell proliferation. Both IL-3 and FL synergized individually with IL-7, whereas combinations of these two cytokines induced an apparently additive effect. These data show that FL stimulates the early stages of B lymphopoiesis, which is a novel activity of this cytokine. Similar activity of FL on murine early B lymphopoiesis was recently reported. On the other hand, other investigators have suggested that IL-7 failed to induce proliferation of early mouse or human B-cell progenitors, even in the presence of stromal cells. The lack of IL-7 responsiveness may be attributed to the fact that the B-cell progenitors used in these studies represented a less differentiated B-cell progenitor population as described...
Fig 4. Phenotypes of cultured pro-B cells. Pro-B cells cultured either in the absence (top panels) or the presence (bottom panels) of 30R, with IL-7 + IL-3 + Fl, were obtained on day 14 or day 21 and analyzed by flow cytometry. For surface staining, propidium iodide-negative events were collected. For clgM staining, events were collected within a lymphoid-blastoid cell gate. Results are shown by 50% log-density contour plots.
The present data furthermore indicate that KL has no effect on the formation by CD34+TDL0- human BM cells or on the proliferation by primitive hematopoietic human progenitor cells,26-28 indicating that FL has a broader range of activities,22-24 proliferation of human pro-B cells in vitro.25-27 B-cell precursors27 and CD34+CD10+ pro-B cells28 have also been reported. Thus, FL and IL-3 are the only two cytokines, which thus far have been shown to augment IL-7-induced proliferation of human pro-B cells in vitro.

FL is produced in many different tissues, including hematopoietic organs,29,30 whereas the expression of its receptor is limited to hematopoietic progenitors.31,32 In contrast, IL-3 is produced by activated T cells.40 Based on this differential production of IL-3 and FL, it may be speculated that FL plays a regulatory role in B lymphopoiesis in the BM under normal physiologic conditions, whereas IL-3 may augment B lymphopoiesis during pathologic conditions, such as acute systemic infections. However, it has to be noted that FL has also costimulatory effects on IL-3- or GM-CSF-induced proliferation by primitive hematopoietic human progenitor cells,33-35 indicating that FL has a broader range of activities, than only stimulating B lymphopoiesis.

The present data furthermore indicate that KL has no effect on human B lymphopoiesis either alone, or together with IL-7, which is in line with previous reports showing that KL had no effect on TdT+ lymphoid progenitor colony formation by CD34+CD10- human BM cells36 or on the expansion of CD19+clgM- cells from cord blood CD34+ cells.34 In contrast, some, but not all,14 studies showed that KL can promote murine B lymphopoiesis,41,42 illustrating that the role of KL on B lymphopoiesis is still controversial.

Although human pro-B cells strongly proliferate in the absence of stromal cells, the addition of a cloned murine stromal cell line, which supports murine B lymphopoiesis in Whitlock-Witte cultures, further promoted the expansion of these cells. Optimal proliferation of human pro-B cells was achieved in cocultures with BM stromal cells supplemented with IL-7 + IL-3 + FL. Under these conditions, generally 40- to 50-fold increases in cellularity (and sometimes even considerably higher) were obtained by the third week of culture, which are extraordinary high levels of proliferation for human B-cell progenitors in vitro. Previous studies have shown that murine BM stromal cells can induce differentiation of human hematopoietic stem cells into both B-lymphoid and myeloiderythroid lineages in vitro.33 Expansion of CD19+clgM- cells from cord blood CD34+ cells was induced in coculture with murine stromal cells in the absence of exogenous cytokines.34 In addition, human acute lymphoblastic leukemia cells with a pre-B cell phenotype were shown to proliferate in the BM of SCID mice.43,44 These findings together with our present results strongly suggest the presence of a species cross-reactive costimulatory molecule(s) expressed by murine BM stromal cells. The increases in cellularity induced by IL-7 + IL-3 + FL in the absence of stromal cells were in the same range as those obtained from cocultures with stromal cells supplemented with IL-7 alone, which suggested that IL-3 and FL could replace the role of stromal cells. However, the enhanced cell expansion induced in the presence of 30R stromal cells and optimal

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Abbreviation: ND, not done.
doses of IL-7, IL-3, and FL indicate that the costimulatory molecule(s) expressed on stromal cells are probably different from IL-7, FL, and KL, although the possibility remains that FL and KL in membrane-bound form may be more physiologic and exert a greater effect. Like 30R, primary human stromal cell cultures were found to further enhance cytokine-driven pro-B cell expansion (Namikawa et al, unpublished results, 1994-1995). However, these human stromal cultures showed significant donor variations, which were probably caused by the heterogeneity of the primary stromal cell populations, and therefore were not investigated further.

The increase in cells expressing cIgM and slgM indicates that IL-7 supports not only the growth but also differentiation of pro-B cells. The acquisition of cIgM and slgM correlated with the loss of CD34 expression on a large proportion of cells. Furthermore, preliminary studies showed that these cultured pro-B cells further differentiated into Ig-producing cells of multiple Ig subclasses including IgM, IgG, IgG4, and IgE after stimulation with anti-CD40 MoAb + IL-4 in secondary cultures (Namikawa et al, unpublished results, 1994-1995), suggesting that the differentiation of pro-B cells to pre-B cells induced in the present culture conditions may reproduce normal differentiation pathways. However, it is presently not clear whether IL-7 actively induces differentiation of pro-B cells or simply supports pro-B cells to undergo their programmed differentiation by supporting their growth. The CD34+CD19+ population is heterogeneous with regard to the status of Ig gene rearrangement, because it contained a minor subpopulation of cIgM+ cells. Therefore, it is also conceivable that only a part of pro-B cells, which reach a certain stage of gene rearrangement, differentiate into cIgM+ stage in response to IL-7, particularly because a significant number of cells remained at the cIgM stage even after 3 to 4 weeks of culture. Further studies will be required to further dissect the functional heterogeneity of CD34+CD19+ pro-B cell population including the CD34+CD19+cIgM+ subset. Similar results suggesting the differentiation-supporting activity of IL-7 on human pro-B cells have been reported.

In this study, IL-7 induced a loss of CD34 and terminal deoxynucleotidyl transferase expression in CD10+CD34+-cIgM+ pro-B cells which correlated with the acquisition of cIgM expression, and downregulation of CD31 expression on these cells cultured in the presence of BM stromal cells. The relative contributions of IL-7 and stromal cells to pro-B cell differentiation could not be elucidated in this study, because both stromal cells and IL-7 were required for pro-B cell expansion and differentiation. However, the present data clearly show that IL-7 alone induces significant increases in cIgM+ cells. More interestingly, stromal cells further enhanced pro-B cell differentiation. Pro-B cells cultured with stromal cells and IL-7 expressed cIgM, as well as slgM more rapidly than those cultured without stromal cells.

Despite their clear B-cell differentiation enhancing effects, stromal cells, on the other hand, were found to support the maintenance and even the expansion of a proportion of CD34+CD19+ pro-B cells. Interestingly, the percentages of CD34+CD19+ cells in cultures with stromal cells were considerably higher than those cultured in the absence of stromal cells. The maintenance of CD34+CD19+ cells in these cultures is likely to contribute to the higher cell yield for longer periods of time in these cultures. The greater recovery of CD34+CD19+ pro-B cells may be caused by the expansion of this initial progenitor population. Alternatively, stromal cells may maintain some of the CD34+CD19+ cells in a quiescent state, thereby preventing the rapid exhaustion of progenitor activity, which would be concordant with a recent observation that CD10+CD34+ cells could be maintained in the presence of human BM stromal cells. Neither IL-3 nor FL could substitute for the stromal-derived activity responsible for the maintenance and expansion of the CD34+CD19+ cell population, further supporting the notion that additional stromal-derived molecule(s) exist.

Thus, stromal cells play two apparently contrasting roles in regulating the differentiation of pro-B cells in vitro; ie, maintenance of the CD34+CD19+ progenitor population and promotion of Ig gene expression. However, this can be explained by the presence of two or more regulatory molecules present on, or produced by, stromal cells acting on different subpopulations of B cells.

Addition of IL-3 and/or FL to the cultures did not further induce or enhance the percentages of CD34+ or Ig expressing cells in IL-7-containing cultures, indicating that these cytokines act to support the proliferation, but not the differentiation, of pro-B cells. Studies to evaluate the effects of other stromal-derived molecules, such as PBSF, recently cloned from a murine BM stromal cell line, IG-1 known to promote the differentiation of murine B cells, or the adhesion molecule VCAM-1, which was suggested to be involved in supporting the proliferation of normal and leukemic human B-cell progenitors, in a stroma-free culture would be particularly interesting for a better understanding of the molecular mechanisms by which stromal cells regulate B-cell development. The present data show that the culture system described herein is useful for the further analysis of the complicated, yet ordered, regulatory mechanisms of human B-cell growth and differentiation supported by the BM microenvironment.

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