Clonal Variation in the B-Lineage Acute Lymphoblastic Leukemia Response to Multiple Cytokines and Bone Marrow Stromal Cells


Department of Laboratory Medicine/Pathology, University of Minnesota Cancer Center, University of Minnesota Medical School, Minneapolis, Minnesota 55455

ABSTRACT

The acquisition of genetic abnormalities in human B-lineage acute lymphoblastic leukemia (ALL) culminates in the clonal expansion of bone marrow (BM)-derived leukemic blasts. However, the response of leukemic cells to signals transduced by the BM microenvironment is not completely understood. The present study describes a new human B-lineage ALL cell line designated BLIN-4 (BLINage-4). BLIN-4 cells respond to multiple cytokines/human BM stromal cell-derived molecules. One subline (BLIN-4E) undergoes cell death in the absence of BM stromal cells or cytokines and slowly proliferates on human BM stromal cells supplemented with interleukin (IL)-7 + FLT3-ligand. Another subline (BLIN-4L) slowly proliferates in the absence of cytokines and BM stromal cells and shows robust proliferation on BM stromal cells supplemented with IL-7 + FLT3-ligand. Although human BM stromal cells are comparable with IL-7 + FLT3-ligand in supporting proliferation of BLIN-4L cells, neutralizing antibody experiments demonstrate that BLIN-4L proliferation on BM stromal cells is IL-7/FLT3-ligand independent. BLIN-4L could also respond to human thymic stromal lymphopoietin. BLIN-4E and BLIN-4L have the identical immunoglobulin heavy chain rearrangement and a CD10+/CD19+/CD20+/CD22+/CD40+ /μ heavy chain phenotype. The original BM leukemic blasts harbored a ring chromosome 4 with a low percentage of cells also having either trisomy 8 or trisomy 18. The BLIN-4 sublines maintained the ring chromosome 4, but the trisomy 8 and trisomy 18 segregated into BLIN-4E and BLIN-4L, respectively. Thus, the BLIN-4 sublines exhibit biological characteristics consistent with a potential evolution in B-lineage ALL involving subclones with decreasing requirements on the BM microenvironment.

INTRODUCTION

Human B-cell development occurs as an ordered progression of maturation steps characterized by prominent checkpoints involving rearrangement and expression of heavy and light chain immunoglobulin genes (reviewed in 1). Although functional immunoglobulin rearrangements leading to expression of the pre-B-cell receptor and B-cell receptor are essential for successful development of B-lineage cells, traversal of these checkpoints is also influenced by the fetal liver and BM microenvironment.

The identity of the survival/proliferation factors that are essential for normal human B-cell development is only partially understood. We have discussed in detail the similar and distinct outcomes of IL-7 signaling on human and murine B-cell precursors (1). IL-7 is a nonredundant BM-derived cytokine that is essential for murine B-cell development (2). This criticality in murine IL-7 signaling is underscored by comparable perturbations in murine B-cell development that accompany mutations in the γc subunit of the receptors for IL-2, 4, 7, 9, and 15 (3, 4), the IL-7 receptor α chain (5), and the Jak3 tyrosine kinase (6, 7). In striking contrast is the presence of normal or even elevated numbers of B cells in X-linked severe combined immunodeficiency patients with mutations in γc (8), Jak3 (9, 10), and the IL-7 receptor α chain (11). Other cytokines such as FLT3-L and IL-3 have been reported to stimulate normal human pro-B cells in vitro (12), but whether either or both could compensate for the absence of IL-7 signaling in the aforementioned immunodeficiency patients is unknown.

One approach that may facilitate identification of BM stromal cell products essential for the proliferation of human B-cell precursors is the use of in vitro human BM stromal cell cultures. We have developed a culture system that uses freshly isolated nontransformed human BM stromal cells established from normal human BM (13, 14). A somewhat similar human BM stromal cell culture system has been used by Manabe et al. (15, 16) and Kumagai et al. (17) to study the apoptotic sensitivities of freshly isolated B-lineage ALL. We have also used this human BM stromal cell culture to establish a human pre-B ALL cell line that requires direct BM stromal cell contact for optimal survival/proliferation (18). The present report describes a new human B-lineage ALL cell line, designated BLIN-4, similarly established by plating freshly isolated leukemic cells on human BM stromal cells. We show that BLIN-4 cells respond to at least four distinct cytokines and unknown human BM stromal cell-derived molecule(s). We also describe sublines of BLIN-4 with differing sensitivities to survival/proliferation signals, suggesting that BLIN-4 may be a model for tumor progression in B-lineage ALL.

MATERIALS AND METHODS

Establishment of BLIN-4. Cryopreserved BM leukemic cells from a 14-year-old female with newly diagnosed B-lineage ALL (CD10+/CD19+/CD7−) were thawed from liquid nitrogen and plated onto a confluent layer of adult BM stromal cells in 96-well flat-bottomed microtiter plates (Costar, Cambridge, MA) in X-VIVO 10 serum-free medium (BioWhittaker, Walkersville, MD). Cultures were initiated at a density of ~3 × 10^6 cells/well. Because human pro-B-cell proliferation on human BM stromal cells is enhanced by addition of exogenous IL-7 (13, 19), the BM leukemic cells were cultured in the absence or presence of 10 ng/ml IL-7 (PeproTech, Rocky Hill, NJ). During the initial 2 months, the cultures were fed twice/week with fresh X-VIVO 10, with or without IL-7. After ~2 months, leukemic cell/BM stromal cell cultures supplemented with IL-7 contained viable lymphoblastic cells as judged by inverted light microscopy. The cellular content of the wells containing viable cells was diluted ~1:4 and passaged onto fresh BM stromal cells supplemented with 10 ng/ml IL-7. After an additional 2 weeks, the leukemic cells were pooled from multiple wells and phenotyped. The leukemic cells established in culture at this stage were designated BLIN-4.

Other Cells. BLIN-1 is a pre-B ALL cell line established in this laboratory (20). BLIN-2 is a pre-B ALL BM stromal cell-dependent cell line established in this laboratory (18). BM stromal cells were established from human fetal BM using methods described previously for establishment and maintenance (18, 21). Fetal BM was obtained from 19- to 21-week gestational age fetuses, in accordance with guidelines established by the University of Minnesota Committee on the Use of Human Subjects in Research. We have shown previously that human foreskin fibroblasts are comparable with human BM

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3 To whom requests for reprints should be addressed, at University of Minnesota Cancer Center, Mayo Mail Code 806, 420 Delaware Street SE, Minneapolis, MN 55455. Phone: (612) 626-1422; Fax: (612) 626-3941; E-mail: lebie001@tc.umn.edu.

3 The abbreviations used are: BM, bone marrow; IL, interleukin; FLT3-L, FLT3-ligand; TSLP, thymic stromal lymphopoietin; ALL, acute lymphoblastic leukemia; mAb, monoclonal antibody; PE, phycoerythrin; BLIN-4; B lineage-4; BLIN-4E, BLIN-4 early; BLIN-4L, BLIN-4 late; IgH, immunoglobulin heavy chain; bFGF, basic fibroblast growth factor; IGF, insulin-like growth factor; OSM, oncostatin M; SCF, stem cell factor; SFDC1a, stromal cell-derived factor 1a.
stromal cells in support of human B-cell development (21). Thus, these two adherent cell populations were used interchangeably in the current study.

**Antibodies and Cytokines.** mAbs recognizing specific immunoglobulin molecules, membrane proteins, and their conjugation to FITC or biotin have been described previously (13, 14, 18, 21). A mAb to the human VpreB subunit of the surrogate light chain was a generous gift from Y-H. Wang and M.D. Cooper, University of Alabama, Birmingham, Alabama (22). PE-conjugated anti-IL-7 receptor α chain (CDw127) was purchased from Immunotech (Westbrook, ME). PE-conjugated anti-FLT3 (CD135) was purchased from Becton Dickinson (Mountain View, CA). Neutralizing mouse antihuman FLT3-L and neutralizing goat antihuman IL-7 were purchased from R&D Systems (Minneapolis, MN). IL-6, IL-7, IL-11, BF, FLT3-L, IGF-I, OSM, SCF, and SDF-1α were purchased from PeproTech. IL-3 was purchased from PeproTech. IL-3 was purchased from R&D Systems. Human recombinant TSLP was a generous gift from John Sims and Stewart Lyman (Immunex Corporation, Seattle, WA). Purified human TSLP contained a COOH-terminal six histidine residue tag followed by a FLAG epitope. A detailed description of the cloning and characterization of human TSLP will be reported elsewhere.  

**Immunofluorescent Staining and Flow Cytometry.** These methods have been described previously (13, 14, 18, 21).

**Proliferation Assays.** Human BM stromal cells or human foreskin fibroblasts were seeded into 96-well flat-bottomed microtiter plates in X-VIVO 10. Once the stromal cells or fibroblasts reached confluence, BLIN-4 cells were added to a concentration of 0.5–2 × 10^5 cells/well in a final volume of 200 μl. Cultures were incubated in a 37°C humidified atmosphere containing 5% CO₂.

**Cytogenetics and FISH.** Metaphase cells were harvested from BLIN-4E or BLIN-4L cells proliferating on BM stromal cells. Metaphase chromosomes were G-banded using Wright stain according to standard cytogenetic methodologies (23). Dual color FISH was conducted using probes specific to the centromeres of chromosome 8 (labeled in spectrum green) and chromosome 18 (labeled in spectrum orange) and chromosome 18 (labeled in spectrum green) according to manufacturer’s instructions (VYSIS, Inc., Downers Grove, IL).

**Southern Blotting.** Southern blotting was conducted as described previously (24) with some modification. Cells were digested at 56°C for 3 h in TE [10 mM Tris (pH 8)-1 mM EDTA], 0.5% SDS, 0.16 mg/ml proteinase K, and 0.08 mg/ml RNase A. DNA was then extracted with equal volumes of phenol: isoamyl alcohol (49:1), phenol:chloroform:isoamyl alcohol (25:24:1), and isoamyl alcohol (49:1), phenol:chloroform:isoamyl alcohol (25:24:1), and chloroform:isoamyl alcohol (24:1). DNA was precipitated in 1/10 volume 3 M sodium acetate and 2.5 volumes of ethanol. DNA was dissolved in 50–100 μl of TE and digested with HindIII (Life Technologies, Inc., Gaithersburg, MD) for 18 h at 37°C. Digested DNA (5–10 μg) was loaded per lane of a 0.8% gel and electrophoresed in TAE (40 mM Tris-acetate and 2.5 volumes of ethanol. DNA was dissolved in 50–100 μl of TE and digested with HindIII (Life Technologies, Inc., Gaithersburg, MD) for 18 h at 37°C. Digested DNA (5–10 μg) was loaded per lane of a 0.8% gel and electrophoresed in TAE (40 mM Tris-acetate and 2.5 volumes of ethanol. DNA was dissolved in 50–100 μl of TE and digested with HindIII (Life Technologies, Inc., Gaithersburg, MD) for 18 h at 37°C. Digested DNA (5–10 μg) was loaded per lane of a 0.8% gel and electrophoresed in TAE (40 mM Tris-acetate and 0.5% SDS, followed by two more washes at 62°C for 10 min in 1 × SSC/0.5% SDS and a final wash for 5 min at room temperature in 2 × SSC. The membrane was then exposed to film for 24 h.

**Statistics.** P values were calculated using the Student’s paired t test.

**RESULTS**

**Establishment of BLIN-4.** We have described previously a cell line (BLIN-2) that requires human BM stromal cells for optimal survival and proliferation (18). We now describe a new cell line, designated BLIN-4, that was also established by plating BM leukemic blasts from a patient with B-lineage ALL onto human BM stromal cells. Following an initial 1-month period of gradual leukemic cell death, BLIN-4 cells could be passaged as a stable cell line after ~2 months in culture. Two sublines of the original parental BLIN-4 cell line were eventually derived designated BLIN-4E and BLIN-4L. The distinction between the two reflects the stochastic emergence of two sublines with different growth characteristics (see below) during the initial 3–6 months in culture and is not meant to imply that BLIN-4L evolved directly from BLIN-4E. Fig. 1 shows that BLIN-4E and BLIN-4L express a qualitatively and quantitatively identical CD10⁺/CD19⁻/CD22⁻/CD40⁻ B-lineage phenotype. The expression of cell surface VpreB on BLIN-4E cells in the absence of μ heavy chain is not without precedent in B-lineage ALL. Neither subline expressed CD7, CD20, CD34, CD80, or CD86. Interestingly, BLIN-4L cells expressed higher levels of CD49d/CD29 (VLA-4) compared with BLIN-4E cells, whereas both sublines expressed identical levels of CD11a/CD18 (LFA-1).

Southern blot analysis with a human Jκ probe indicated that BLIN-4E and BLIN-4L sublines both harbored a single ~8–kb rearrangement at the IgH locus with the other allele deleted (Fig. 2). Unfortunately, DNA was not available from the diagnostic BM specimen, so we are unable to characterize the IgH rearrangements in the original leukemic blasts. Cytogenetic analysis of the original BM specimen and the BLIN-4 sublines revealed multiple related clones that included 46,XX,r(4)/47,XX,r(4),+18/47,XX,r(4),+8/and 48,XX,r(4),+8,+18. Because of the low yield of mitotic cells in the original BM aspirate and early passages of BLIN-4, we were unable to accurately estimate the relative frequencies of the karyotypically distinct subclones by G-banding analysis. Therefore, we performed FISH using chromosome 8- and 18-specific probes. Analysis of 200 interphase cells in the original BM specimen revealed that trisomy 8 was present in 11.5% of cells, and trisomy 18 was present in 2.5% of

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S. Lyman, personal communication.
cells. Analysis of 300 interphase BLIN-4E cells revealed that 89% of the cells had a trisomy 8, and 0% had a trisomy 18. In marked contrast, analysis of 300 interphase BLIN-4L cells indicated that 88% had trisomy 18, and 1% had a trisomy 8. Fig. 3 shows representative FISH profiles of the two sublines.

**BM Stromal Cell Dependency of BLIN-4 Sublines.** Approximately 10 months following initial plating, BLIN-4 cells slowly proliferated on human BM stromal cells and had a doubling time of ~10 days (Fig. 4). BLIN-4 cells slowly died in the absence of BM stromal cells, and IL-7 marginally delayed cell death. Addition of IL-7 at day 0 significantly enhanced (P < 0.001) proliferation of BLIN-4 cells on BM stromal cells between days 7 and 15 (Fig. 4). Substitution of FLT3-L for IL-7 gave similar results (data not shown). Direct contact with BM stromal cells was necessary for optimal proliferation, because only ~25% of contact-dependent proliferation remained when BLIN-4 cells were incubated in Transwell inserts above the BM stromal cell monolayer (data not shown).

Approximately 10 months after initiating the BLIN-4 cell line, we noticed that the cells were exhibiting enhanced survival in X-VIVO 10 serum-free medium alone. We designated this subline BLIN-4L to distinguish it from the early passaged subline (BLIN-4E) with more stringent culture requirements. Table 1 shows the results of two experiments comparing the survival/proliferation characteristics of BLIN-4E and BLIN-4L. In these experiments skin fibroblasts were used in lieu of BM stromal cells as an adherent cell monolayer. Consistent with the results shown in Fig. 4, BLIN-4E cells were >90% dead by day 7 in the absence of cytokines and/or fibroblasts. Inclusion of IL-7 and FLT3-L only marginally inhibited cell death. BLIN-4E cells essentially survived for 2 weeks when cultured on skin fibroblasts. Addition of IL-7 and FLT3-L to the fibroblasts led to a significant enhancement in BLIN-4E cell number compared with BLIN-4E cells maintained on fibroblasts alone (P = < 0.001). However, in the presence of exogenous IL-7/FLT3-L and skin fibroblasts, BLIN-4E cells only expanded 4- to 5-fold by day 14 (Table 1). In marked contrast, BLIN-4L cells proliferated in medium alone, and proliferation was comparably enhanced by culturing in either IL-7/FLT3-L or on skin fibroblasts. Maximum expansion occurred when BLIN-4L cells were cultured in the presence of IL-7/FLT3-L and skin fibroblasts, with 90- to 260-fold increases in cell number by day 14. These collective results indicate that BLIN-4L cells are dramatically more sensitive to proliferation signals transduced by cytokines and fibroblast adherent layers than are BLIN-4E cells.

As noted above, BLIN-4L expressed higher levels of VLA-4 than BLIN-4E (Fig. 1). Consistent with this quantitative difference, BLIN-4L cells were approximately twice as adherent to BM stromal cells as BLIN-4E cells when measured in static (nonfluid shear) adhesion assays (data not shown). However, the adherence of BLIN-4L cells to BM stromal cells was completely blocked by a mAb...
to the CD29/β1 subunit, whereas the adherence of BLIN-4E cells was not.

**Cytokine Responsiveness of BLIN-4L.** The capacity of BM stromal cells to support the proliferation of BLIN-4L cells could be partially substituted by conditioned medium from BM stromal cells (data not shown). We therefore screened a panel of cytokines shown previously to stimulate human or murine B-cell precursors in other experimental systems. The panel included: IL-3, IL-6, IL-11, bFGF, FLT3-L, IGF-I, OSM, SCF, and SDF-1α. As expected, IL-7 enhanced proliferation of BLIN-4L cells (Fig. 5). The pool of cytokines (represented by IL-7 + IL-3, IL-6, IL-11, bFGF, FLT3-L, IGF-I, OSM, SCF, and SDF-1α) promoted an additional 3-fold increase over IL-7 alone. When individual components of the cytokine panel were tested for their capacity to cooperate with IL-7, FLT3-L was the only cytokine that promoted the proliferation of BLIN-4L cells (Fig. 5). Additional results in Fig. 6 demonstrated that BLIN-4L cells exhibited a dose-dependent response to FLT3-L and IL-7. Note that the data in Fig. 6 represent survival/proliferation at day 7 only. Thus, inclusion of 10 ng/ml IL-7 resulted in a 63% increase in BLIN-4L cell number at day 7 (compared with day 0 input), and inclusion of 10 ng/ml FLT3-L resulted in a 31% increase in BLIN-4L cell number at day 7 (compared with day 0 input). However, inclusion of 10 ng/ml of both cytokines led to a synergistic 352% increase in BLIN-4L cell number at day 7 (compared with day 0 input).

We have reported previously (14) that human BM stromal cells, which constitute the supportive microenvironment in our culture system, produce extremely small amounts of IL-7 (1.0–2.0 pg/ml). However, this quantity of IL-7 (in whatever physical disposition it exists in our BM stromal cell culture system) has no bioactivity for normal B-cell precursors (14). FLT3-L has been reported to be produced by human BM stromal cells (25). We confirmed that report by finding that fetal BM stromal cells used in the current study produced 10 ng/ml of FLT3-L as measured by ELISA (data not shown). We therefore examined whether the endogenous production of IL-7 and/or FLT3-L contributed to the survival and proliferation of BLIN-4L cells. This was accomplished by determining whether neutralizing antibodies against FLT3-L and IL-7 would inhibit the proliferation of BLIN-4L cells. As shown in Fig. 7, we first demonstrated the efficacy of the neutralizing antibodies to inhibit the stimulatory activity of exogenous FLT3-L and IL-7. The FLT3-L and IL-7 responses were completely blocked by neutralizing anti-FLT3-L and neutralizing anti-IL-7, respectively. Normal mouse IgG (control for anti-FLT3-L) and normal goat IgG (control for anti-IL-7) had no effect. Importantly, the combined stimulus of FLT3-L + IL-7 was completely blocked by anti-FLT3-L + anti-IL-7. Knowing that the neutralizing antibodies could inhibit >99% of exogenous FLT3-L and IL-7 bioactivity, we then tested their capacity to inhibit BLIN-4L proliferation on BM stromal cells. The results at the bottom of Fig. 7 show that inclusion of anti-FLT3-L, anti-IL-7, or anti-FLT3-L + anti-IL-7 had absolutely no affect on BLIN-4L proliferation on BM stromal cells.

**Cytokine Receptor Expression and TSLP Responsiveness of BLIN-4E and BLIN-4L.** The responsiveness of BLIN-4L cells to IL-7 and FLT3-L was corroborated by the detection of the IL-7 receptor α chain and FLT3 on the cell surface (Fig. 8). The level of IL-7 receptor α chain and FLT3 on BLIN-4E cells was barely above background (Fig. 8) but likely to be physiologically significant because BLIN-4E survival/proliferation was enhanced by IL-7 and FLT3-L (Fig. 4 and Table 1).

A recently described murine cytokine designated TSLP has been shown to enhance proliferation and development of murine B-lineage cells and factor-dependent cell lines (26–30). TSLP signaling requires the IL-7 receptor α chain and a newly described hematopoietin receptor (30–33). Because BLIN-4E and BLIN-4L both express the IL-7 receptor α chain (Fig. 8), we tested their response to human TSLP in the presence or absence of BM stromal cells. As shown in Fig. 9, BLIN-4L cells exhibited a dose-dependent response to human TSLP at concentrations ranging from 0.1–100 ng/ml. The two highest concentrations of TSLP enhanced the expansion of BLIN-4L by 1.9- and 2.1-fold at day 21, compared with medium alone. However, the TSLP effect was considerably weaker than the effect of BM stromal cells, which supported a 142-fold increase in BLIN-4L cell number over 21 days. In contrast, BLIN-4E cell survival was not influenced by TSLP (Fig. 9). Neither BLIN-4E or BLIN-4L responded to TSLP in the presence of BM stromal cells.

**DISCUSSION**

The complete identification and characterization of human BM stromal cell-derived molecule(s) that transduce survival/proliferation signals essential for normal human B-cell development remains to be accomplished. We have recently reviewed the role of IL-7 and other cytokines in regulating the survival, proliferation, and differentiation of normal human B-cell precursors (1). IL-7 exerts weak-to-modest proliferative effects on normal human B-cell precursors in the pres-

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**Table 1 Growth properties of BLIN-4E and BLIN-4L**

<table>
<thead>
<tr>
<th></th>
<th>BLIN-4E EXP 1</th>
<th>BLIN-4E EXP 2</th>
<th>BLIN-4L EXP 1</th>
<th>BLIN-4L EXP 2</th>
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<tr>
<td>DAY 7</td>
<td>0.3 ± 0.1 (1.0)</td>
<td>0.2 ± 0.1 (0.1)</td>
<td>0.4 ± 0.1 (0.1)</td>
<td>0.1 ± 0.0 (0.0)</td>
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<tr>
<td>DAY 14</td>
<td>4.9 ± 0.7 (2.4)</td>
<td>2.2 ± 0.5 (1.1)</td>
<td>15.4 ± 0.3 (3.4)</td>
<td>46.5 ± 1.5 (10.3)</td>
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<tr>
<td>IL-7/FLT3-L</td>
<td>2.3 ± 0.0 (0.7)</td>
<td>1.2 ± 0.1 (0.4)</td>
<td>4.2 ± 0.6 (0.4)</td>
<td>0.4 ± 0.1 (0.1)</td>
</tr>
<tr>
<td>FIBRO</td>
<td>6.1 ± 0.5 (2.0)</td>
<td>6.6 ± 0.4 (2.1)</td>
<td>7.3 ± 0.4 (1.1)</td>
<td>4.6 ± 0.3 (0.7)</td>
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<tr>
<td>FIBRO + IL-7/FLT3-L</td>
<td>8.2 ± 0.6 (2.6)</td>
<td>15.9 ± 0.9 (5.1)</td>
<td>14.3 ± 1.3 (2.2)</td>
<td>26.6 ± 3.9 (4.1)</td>
</tr>
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</table>

* Data points are expressed as total cell number per well (×10⁴) and represent the mean ± SD of triplicate wells. Values in parentheses represent fold-increase in cell number compared to day 0 input.
* IL-7 and FLT3-L were each used at 10 ng/ml.
ence of BM stromal cells (12, 13, 19) but is at most a survival signal for normal human B-lineage cells. However, IL-7 signaling regulates gene expression in normal human B-cell precursors (34, 35), which may occur through pathways biochemically separable from classical survival/proliferation pathways mediated by PI-3 kinase and Ras. Nonetheless, the normalcy of human B-cell development in the absence of an IL-7 activated signaling pathway in vivo (8 –11) and in vitro (14) indicates that other cytokines must play a critical role.

The BLIN-4 cell line was established as part of an effort to develop cellular tools for elucidating the influence of the BM microenvironment on normal and leukemic B-cell development. Following the establishment of BLIN-4 as a stable cell line that could be continuously passaged on human BM stromal cells (or foreskin fibroblasts), we observed the emergence of sublines with differing dependencies on adherent cells and/or cytokines for survival and proliferation (Table 1). Fig. 1 shows that BLIN-4E and BLIN-4L are remarkably similar in quantitative expression of cell surface molecules with two exceptions. BLIN-4E expressed low but detectable levels of cell surface VpreB in the absence of μ heavy chains. VpreB staining on BLIN-4L cells (Fig. 1) showed a very subtle shift, but this was not a reproducible result. Human B-lineage ALL cells expressing VpreB associated with unknown proteins of Mr 105,000 and Mr 130,000 have been described (36). However, these complexes have not been biochemically identified on normal human pro-B cells (1). The physiological significance of the differential expression of cell surface VpreB on BLIN-4E and BLIN-4L is unknown. The second difference was the higher level of CD49d/α4 and CD29/β1 subunits of VLA-4 on BLIN-4L (Fig. 1). The higher level of VLA-4 correlated with a higher degree of adhesion to BM stromal cells by BLIN-4L compared with BLIN-4E. Moreover, mAb to the β1 subunit of VLA-4 com-
bFGF, IGF-I, OSM, SCF, and SDF-1 bodies to IL-7 and FLT3-L had no effect on survival/proliferation of BLIN-4 is unknown. Neutralizing anti-IL-7 and FLT3-L had no effect on survival/proliferation of BLIN-4 cells. Neutralizing anti-IL-7 and FLT3-L (Fig. 7). As with IL-7, it is conceivable that the culture conditions we used to establish and maintain human BM stromal cells do not adequately support FLT3-L/FLT3 interactions.

The identity of the human BM stromal cell-derived molecule(s) that promote the proliferation of BLIN-4 is unknown. Neutralizing antibodies to IL-7 and FLT3-L had no effect on survival/proliferation of BLIN-4L cells (Fig. 7). Furthermore, inclusion of IL-3, IL-6, IL-11, bFGF, IGF-I, OSM, SCF, and SDF-1α had no effect on BLIN-4L cell survival/proliferation in the absence of BM stromal cells (Fig. 5). It is therefore likely that an unknown cytokine/chemokine secreted by or cleaved from BM stromal cells and potentially associated with the extracellular matrix plays a critical role in promoting the survival/proliferation of BLIN-4 cells. An obvious question is whether human TSLP is involved. The full sequence of human TSLP has not been published nor, to our knowledge, have antibodies specific for human TSLP been produced. Whether the human BM stromal cells used in our culture system produce biologically active TSLP will be an important question to resolve in future studies. However, it is unlikely that TSLP could account for the complete effect of intact BM stromal cells given the difference in the potency between the two stimuli (Fig. 9).

FLT3 is a single-chain class III receptor tyrosine kinase (42), whereas the IL-7 receptor α chain is a member of the hematopoietin superfamily (43). Signaling pathways activated following FLT3-L and IL-7 stimulation have been described (41, 44 and references therein). FLT3 activation in human B-lineage cells induces tyrosine phosphorylation of the SHC adaptor protein and an unidentified p115 (45). Studies using the murine pro-B-cell line BaF3 transfected with full-length human FLT3 indicated that FLT3-L stimulation induces FLT3 to associate with Grb2, resulting in tyrosine phosphorylation of SHC and SHP-2 (46). IL-7 signaling in human B-lineage cells leads to activation of PI-3 kinase (47), STAT1, and STAT5 (48). The synergistic response of BLIN-4 cells to IL-7 + FLT3-L (Fig. 6) suggests that cooperative signaling occurs. It is therefore noteworthy that FLT3-L and IL-7 both induce phosphorylation of the CBL-related protein CBLB in a human B-lineage ALL cell line (49), suggesting that signaling through the two receptors may converge on this component of tyrosine kinase-activated signaling cascades. Studies are in progress to determine the signaling pathways activated by FLT3-L, IL-7, and TSLP in BLIN-4L.

B-lineage ALL cell lines have been produced in a number of laboratories (50 and references therein), but the vast majority exhibit no specific growth factor requirements beyond standard tissue culture medium supplemented with FBS. The BLIN-4 cell lines comprise a clonally related population of cells with distinct differences in survival/proliferation signaling requirements. BLIN-4E and BLIN-4L have the same IgH rearrangement (Fig. 2) but strikingly different sensitivities to cytokine/BM stromal-cell signaling (Table 1). The relationship between the trisomy 8 and trisomy 18 status of the two sublines and their differing sensitivities to cytokine and BM stromal-cell signaling is unknown. Data in Table 1 suggest that BLIN-4L has a heightened sensitivity to all of the cytokine/BM stromal cell signals. The combination of a trisomy 18 and a ring chromosome 4 may underlie this heightened sensitivity by an unknown mechanism. It is also conceivable that the segregation of trisomy 8 and trisomy 18 into BLIN-4E and BLIN-4L is coincidental and has no direct bearing on the biological differences between the two sublines. BLIN-4L may also have acquired mutations in genes with critical functions in regulating a response to cytokine/BM stromal cell signals. An obvious candidate would be Ras, but we found no mutations in codons 12, 13, and 61 of N-Ras and K-Ras in either subline.5 Future studies will focus on identifying the mutations that underlie the biological differences between BLIN-4E and BLIN-4L and how these mutations contribute to loss of BM stromal cell dependency.

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5 T. W. LeBien and J. Perentesis, unpublished observations.

Fig. 9. BLIN-4L cells but not BLIN-4E cells respond to human TSLP. Cells were plated at 4 x 10⁴/ml in X-VIVO 10 supplemented with TSLP or on BM stromal cells in X-VIVO 10 supplemented with TSLP. Cell numbers were quantified on days 7, 14, and 21. Each symbol represents the mean of triplicate values; bars, ± SD. TSLP concentrations in ng/ml were: □, 0; ○, 0.1; ○, 1; △, 10; and ■, 100. This experiment is representative of three independent experiments.
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