Theflt3 Ligand Supports Proliferation of Lymphohematopoietic Progenitors and Early B-Lymphoid Progenitors

By Fumiya Hirayama, Stewart D. Lyman, Steven C. Clark, and Makio Ogawa

We have examined the effects of the murine ligand (FL) for the ftlt3/flk2 tyrosine kinase receptor on the proliferation of murine lymphohematopoietic progenitors as well as committed myeloid and B-cell progenitors. In the presence of erythropoietin, FL alone supported scant colony formation from enriched marrow cells of normal mice. However, when it was combined with interleukin-3 (IL-3), steel factor (SF), or IL-11, FL significantly enhanced colony formation. When tested on enriched marrow cells from 5-fluourouracil (5-FU)-treated mice, FL neither enhanced IL-3-dependent colony formation nor synergized with SF in support of colony formation. However, FL synergized with IL-6, IL-11, or granulocyte-colony stimulating factor (G-CSF) in support of formation of various types of colonies, including multilineage colonies. Approximately 30% of these colonies yielded pre-B-cell colonies when replated in secondary cultures containing SF and IL-7, indicating that 2-cytokine combinations, including FL and IL-6, IL-11, or G-CSF can support the proliferation of primitive lymphohematopoietic progenitors. FL, by itself and in synergy with IL-7 or SF, supported the proliferation of B-cell progenitors. These results show that FL has a wide range of activities in early hematopoiesis and B lymphopoiesis.

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The RECEPTOR-TYPE tyrosine kinases comprise a large family of proteins that are important in the proliferation and differentiation of a number of cells. Among them, c-fms,1 c-kit,2-4 flt3,5 and flk26 are grouped into a subfamily based on organization and sequence similarities. c-fms, a receptor for colony-stimulating factor 1 (CSF-1) or macrophage CSF, is involved in the proliferation and differentiation of macrophage precursors and is required for the survival, proliferation, and differentiation of mononuclear phagocytes, including osteoclasts.2-9 c-kit appears essential for the development of melanoblasts, germ cells, and hematopoietic cells. The W and Sl/loci encode c-kit and its ligand, steel factor (SF), respectively.10-13 And mutations in these genes cause white spotting, infertility, and severe defects in the hematopoietic system.14-15 c-kit and SF also play important roles in survival, proliferation, and differentiation of primitive lymphohematopoietic progenitor16,17 as well as in early B and T lymphopoiesis.18,19 Matthews et al20 attempted to identify hematopoietic stem cell–specific genes that encode tyrosine kinase–type receptors and have cloned two cDNAs, designated fetal liver kinase 1 (flk1) and flk2 from fetal liver cells that were enriched for hematopoietic stem and progenitor populations. flk2 is nearly identical to flt3, which was independently cloned from murine placent.a and also from a population of cells that are phenotypically early T-cell progenitors (CD3−, CD4−, and CD8−) derived from a day 13 murine fetal liver.24 flt3 and flk2 are nearly identical in amino acid sequences except for two amino acids in the extracytoplasmic domain and 51 amino acids near their C termini in the cytoplasmic domain.24 It is likely that they are encoded by one gene and share the same ligand.24 The human homolog of murine flt3/flk2 was cloned from both a pre-B-cell line25 and bone marrow cells that were enriched for hematopoietic stem cells (stem cell tyrosine kinase 1, STK-26). Because of their expression by primitive hematopoietic progenitors26 and lymphoid progenitors25,26 and their structural similarity to c-fms and c-kit, it has been speculated that flt3/flk2 may be important in early lymphohematopoiesis. This hypothesis was in part supported by a study showing that antisense oligonucleotides directed against the STK-1 sequence inhibited hematopoiesis in vitro.26

Recently cDNAs of murine and human ligands (FL) for flt3/flk2 have been cloned.27-29 Here we report that murine FL can support proliferation of primitive murine lymphohematopoietic progenitors in synergy with interleukin-6 (IL-6), IL-11, or granulocyte colony-stimulating factor (G-CSF) and that FL can also support proliferation of B-cell progenitors alone and in combination with SF or IL-7.

MATERIALS AND METHODS

Growth factors. Recombinant, soluble murine FL was expressed in yeast and purified as previously described.27 Purified recombinant human IL-1α was provided by Dr Hirai (Otsuka Pharmaceutical Co, Ltd, Tokushima, Japan). Purified recombinant murine IL-3 was purchased from R & D Systems (Minneapolis, MN). The medium conditioned by Chinese hamster ovary (CHO) cells that have been genetically engineered to produce murine IL-3 was a gift from T. Sudo of Biomaterial Research Institute (Yokohama, Japan). Purified recombinant murine IL-4 and human IL-7 were provided by C. Faltynek of Sterling Drug (Malvern, PA). Purified recombinant human IL-6 was a gift from M. Naruto of Toray Industries (Yokohama, Japan). Purified recombinant human IL-11 and murine IL-12 were gifts of P. Schendel and S.F. Wolf of the Genetics Institute, Cambridge, MA, respectively. Purified recombinant murine SF was provided by Kirin Brewery Co (Tokyo, Japan). Conditioned medium (CM) of CHO cells that had been transfected with an expression plasmid containing murine SF cDNA was provided by D. Donaldson of the Genetics Institute. Purified bacterially derived human G-CSF was provided by Dr L. Sousa (Amgen, Thousand Oaks, CA). Purified recombinant human erythropoietin (Epo) was provided by the Genetics Institute Clinical Manufacturing Group. Serum-free CM of pokeweed mitogen–stimulated spleen cells (PWM-SCM) was prepared as described.30 Unless specified otherwise, CHO cell CM (CHO-
cytokines used were as follows: IL-1α, 1 ng/mL; IL-3 (purified), 10 ng/mL; IL-3 (CHO-CM), 200 U/mL; IL-4, 10 ng/mL; IL-6, 100 ng/mL; IL-7, 5 ng/mL; IL-11, 100 ng/mL; IL-12, 10 ng/mL; SF (purified), 100 ng/mL; SF (CHO-CM), 5 U/mL; G-CSF, 1 ng/mL; Ep, 2 U/mL; PWM-SCM, 5% (vol/vol).

**Progenitor purification.** Bone marrow cells were harvested from 10- to 16-week-old normal BDF1 mice (Charles River, Raleigh, NC) and from mice injected intravenously with 5-fluorouracil (5-FU; Adria Laboratories, Colombus, OH) at 150 mg/kg (body weight) 2 days before. The marrow cells from normal and 5-FU–treated mice were enriched for progenitors using the technique we described previously. Briefly, cells at densities ranging from 1.063 to 1.077 g/mL were processed with metrizamide (Accurate Chemical & Scientific, Westbury, NY) density centrifugation, and cells expressing lineage-specific antigens were removed by negative immunomagnetic bead selection using a cocktail of monoclonal antibodies (MoAbs) and Dynabeads M-450 sheep-antirat IgG (Dynal, Great Neck, NY). The MoAbs used were anti-B220 (14.8), anti-Mac-1 (M170), anti-Gr-1 (RB6-8C5), anti-L34T4 (OKI.5), anti-Ly-2 (53-6.72) and TER-119. The density-separated, lineage-negative (Lin- ) cells were then stained with fluorescein isothiocyanate (FITC)-conjugated anti-Ly-6A/E (D7) and sorted for Ly-6A/E+ cells using a FACStar Plus cell sorter (Becton Dickinson, Mountain View, CA). In some experiments, Lin- cells were stained with FITC- and PE-conjugated anti-CD43 (S7, PharMingen, San Diego, CA) and then sorted for Ly-6A/E+CD43+ cells, in which lymphohematopoietic progenitor cells are 2-fold further enriched relative to Ly-6A/E+ cells. FITC- and PE-conjugated rat IgG2a (Caltag Laboratories, Inc, South San Francisco, CA) were both used as isotype controls.

**Conal myeloid cell culture.** One hundred Lin- Ly-6A/E+ marrow cells from normal mice and 50 marrow cells from 5-FU–treated mice were plated in 35-mm suspension culture dishes (Becton Dickinson Labware, Lincoln Park, NJ). The culture medium consisted of α medium (Flow Laboratories, Rockville, MD), 1.2% 1500-centi- poise methylcellulose (Shinetsu Chemical, Tokyo, Japan), 25% fetal calf serum (FCS; Intergen, Purchase, NY), 1% denatured fraction V bovine serum albumin (BSA, Sigma Chemical Co, St Louis, MO), 0.1 mmol/L 2-mercaptoethanol (2-ME, Sigma), Epo, and designated growth factors. Dishes were incubated at 37°C in a humidified atmosphere flushed with 5% CO2. Colonies consisting of 50 or more cells were scored on day 13 of incubation. Colonies were identified by the criteria described previously.

**Analysis of myeloid and B-lymphoid potentials of the progenitors.** Study of myeloid and B-lymphoid potentials of progenitors was performed using 3 minor modifications of the two-step methylcellulose culture system described previously. First, 150 Lin- Ly-6A/E+ cells from 5-FU–treated mice were plated in primary methylcellulose culture containing α medium, 1.2% methylcellulose, 25% FCS, 1% BSA, 0.1 mmol/L 2-ME, FL, Epo, IL-7, and either IL-3, IL-11, or G-CSF. On day 11 of culture, 24 or 36 primary colonies were individually picked, washed, and aliquots of cells from each primary colony were replated in secondary methylcellulose culture containing SF and IL-7 for assessment of pre-B-cell colony formation. The number of pre-B-cell colonies was scored on day 10 of culture, after total colony numbers were scored, 30 primary colonies were picked, pooled, and washed. Aliquots of 1/60 of the pooled cells were then replated in secondary pre-B-cell colony culture containing SF and IL-7.

**Second, to test the direct effects of growth factors, Lin- Ly-6A/E+ cells from 5-FU–treated mice were individually plated by micromanipulation in primary culture containing FL and either IL-6, IL-11, or G-CSF. Epo and IL-7 were omitted. On day 11 or 12, the primary colonies were individually picked, washed, and aliquots of each primary colony were replated in secondary pre-B-cell colony culture containing SF and IL-7. Aliquots of cells from each primary colony were simultaneously recultured in two different myeloid suspension cultures in 96-well plates. One was performed in the presence of FL, Epo, and IL-6, IL-11, or G-CSF, depending on which factor was used in the primary culture. The other one was performed in the presence of IL-3, PWM-SCM, and Epo. Myeloid lineage expression was examined cytologically every 2 to 3 days after the reculture.

**Third, to test the inhibitory effects of IL-3 and IL-1α on the B-lymphoid potential of progenitor cells, 150 Lin- Ly-6A/E+ post-5-FU marrow cells were plated in methylcellulose cultured with FL and IL-6, IL-11, or G-CSF in the presence or absence of IL-3 and IL-1α. On day 10 of culture, after total colony numbers were scored, 30 primary colonies were picked, pooled, and washed. Aliquots of 1/60 of the pooled cells were then replated in secondary pre-B-cell colony culture containing SF and IL-7.

**To examine in more detail the effects of FL on colony formation from normal marrow cells:** First we examined the effects of FL, as a single factor or in combination with other cytokines, on colony formation from Lin- Ly-6A/E+ cells of normal mice. The results presented in Table 1 are representative of three experiments. As single agents, FL, SF, and IL-11 supported formation of a small number of granulocyte-macrophage (GM) colonies. IL-3 supported formation of more colonies, including multilineage colonies, than other single factors. When FL and IL-3 were combined, the total colony formation was significantly enhanced. Similarly, synergy was observed between FL and SF and between FL and IL-11 for GM colony formation. Interestingly, many of the colonies supported by FL and IL-11 contained a number of immature cells, therefore it was not possible to determine the colony types in situ.
Table 1. Effects of FL on Lin-Ly-6A/E+ Cells From Normal Marrow

<table>
<thead>
<tr>
<th></th>
<th>GM</th>
<th>GEM</th>
<th>GMM</th>
<th>GEMM</th>
<th>Bi</th>
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<tr>
<td>None</td>
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<tr>
<td>FL</td>
<td>3 ± 1</td>
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<tr>
<td>IL-3</td>
<td>29 ± 1*</td>
<td>0 ± 1</td>
<td>1 ± 1</td>
<td>3 ± 1</td>
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<td>SF</td>
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<tr>
<td>IL-11</td>
<td>5 ± 3</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FL, IL-3</td>
<td>54 ± 3*</td>
<td>0 ± 1</td>
<td>4 ± 1</td>
<td></td>
<td></td>
<td>59 ± 4*</td>
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<tr>
<td>FL, SF</td>
<td>24 ± 71</td>
<td>0 ± 1</td>
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<td>FL, IL-11</td>
<td>48 ± 41*</td>
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</table>

100 Lin-Ly-6A/E+ cells from normal marrow were cultured in the presence of Ep and designated factors. Colony number was counted on day 13.

* Difference between the two groups is significant at P < .001 by Student's t-test.
† Difference is significant at P < .005.
‡ Difference is significant at P < .001.
§ In situ diagnosis was not done.

Table 2. Effects of FL on Murine Post-5-FU Lin-Ly-6A/E+ Cells

<table>
<thead>
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<th></th>
<th>GM</th>
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<th>GMM</th>
<th>GEMM</th>
<th>M</th>
<th>Total</th>
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<tr>
<td>IL-3</td>
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<td>2 ± 1</td>
<td>8 ± 3</td>
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<tr>
<td>FL, SF</td>
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<tr>
<td>FL, IL-3</td>
<td>7 ± 2</td>
<td>1 ± 1</td>
<td>2 ± 1</td>
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<td>2 ± 1</td>
<td>11 ± 4</td>
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<td>FL, IL-4</td>
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<td>IL-11, SF</td>
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<td>5 ± 2</td>
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<td>14 ± 5</td>
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<td>IL-11, IL-3</td>
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<td>19 ± 1</td>
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<td>IL-11, IL-4</td>
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<td>16 ± 6</td>
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<tr>
<td>FL, IL-6</td>
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<tr>
<td>FL, IL-11</td>
<td>7 ± 2†</td>
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</tr>
<tr>
<td>FL, G-CSF</td>
<td>3 ± 2†</td>
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<tr>
<td>FL, IL-12</td>
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<tr>
<td>SF, IL-6</td>
<td>8 ± 4</td>
<td>1 ± 1</td>
<td>6 ± 0</td>
<td>0 ± 1</td>
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<td>16 ± 4</td>
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<tr>
<td>SF, IL-11</td>
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<td>6 ± 0</td>
<td>0 ± 1</td>
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<td>15 ± 2</td>
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<tr>
<td>SF, G-CSF</td>
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<td>3 ± 2</td>
<td>0 ± 1</td>
<td></td>
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<td>10 ± 3</td>
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<tr>
<td>SF, IL-12</td>
<td>2 ± 2</td>
<td>0 ± 1</td>
<td>0 ± 1</td>
<td></td>
<td></td>
<td>3 ± 2</td>
</tr>
</tbody>
</table>

Fifty enriched cells were plated per dish in the presence of Ep and designated factors. Colonies were scored on day 13. SF, IL-4, IL-6, IL-11, G-CSF, and IL-12 as single agents did not support colony formation. Purified recombinant IL-3 was used in this experiment.

† In situ diagnosis was not done.
* Difference is significant at P < .005 by Student's t-test.
‡ Difference is significant at P < .005.
§ Difference is significant at P < .001.

11, IL-12, and G-CSF. None of the individual factors except IL-3 supported colony formation. FL did not synergize with SF, IL-3, or IL-4. The lack of synergy between FL and IL-3 and between FL and SF was in contrast to the synergy between IL-11 and SF, IL-3, or IL-4 in support of colony formation including granulocyte/erythrocyte/macrophage/megakaryocyte (GEMM) colonies and to the studies with normal marrow cells shown in Table 1. Similar to SF, FL enhanced colony formation when combined with either IL-6, IL-11, or G-CSF. However, the number of colonies was significantly smaller than that supported by SF-containing combinations, and FL did not synergize with IL-12. Upon cytologic examination, many colonies supported by FL plus one of IL-11, IL-6, and G-CSF again showed the presence of blast cells and no erythroid or megakaryocytic elements. Therefore, accurate in situ identification of colony types was not possible.

Lineage expression of colonies supported by FL. In culture of both normal and post-5-FU marrow cells, it was not possible to identify colony types in situ on day 13 of culture because of persistence of blast cells. To study if this is due to delayed differentiation and maturation of the colonies, we extended the culture period up to 30 days and performed serial cytologic examination of the cultures. We plated 150 Lin-Ly-6A/E+ post-5-FU marrow cells in the presence of FL, Epo, IL-7, IL-6, IL-11, or G-CSF. On day 11 of culture, we randomly picked 24 or 36 colonies and replated aliquots of the colonies individually in 96-well plates without methylcellulose in the presence of FL, Epo, and either IL-6, IL-11, or G-CSF, depending on which factor was used in the primary culture. Every 2 to 3 days after reculture until day 30, small samples of each culture were procured for cytologic assessment of the myeloid potentials of the progenitors. Simultaneously, aliquots of each primary colony were recultured in methylcellulose in the presence of SF and IL-7 to test the ability to form pre-B–cell colonies. The cumulative myeloid and B-lymphoid lineage expression of the progenitors that yielded sufficient number of cells for the analysis are shown in Table 3. The data are representative of three experiments. Various combinations of myeloid lineages were seen. Most colonies showed only mature myeloid cells by day 26 of culture. In two secondary myeloid cultures supported by FL, IL-11, and Epo, blast cells still predominated even on day 30. Approximately one third of the primary colonies yielded pre-B–cell colonies. There were no obvious differences in the myeloid potentials between colonies containing B-lymphoid potential and those showing no lymphoid potential.
Myeloid and B-lymphoid potentials of individual progenitors. To confirm that the observed effects of FL are direct, we plated individual progenitor cells in culture by micromanipulation. In this experiment, we used Lin- Ly-6A/E+ CD43* post-5-FU marrow cells. Enriched marrow cells were individually plated in methylcellulose culture containing FL and either IL-6, IL-11, or G-CSF. Epo and IL-7 were omitted. On day 10 or 12 of culture, primary colonies were individually picked and aliquots of each primary colony were tested for B-lymphoid and myeloid potentials in secondary pre-B-cell colony and myeloid suspension cultures. Results are summarized in Table 4. Of a total of 105 isolated cells cultured with FL and IL-6, 24 cells (23%) yielded primary colonies, of which 4 (17%) gave rise to pre-B-cell colonies in the secondary culture. Similarly, in the presence of FL and IL-11, 12 of 98 isolated cells (12%) yielded primary colonies, 17% of which (2 of 12) gave rise to pre-B-cell colonies. And in the presence of FL and G-CSF, 6 of 49 cells (12%) yielded primary colonies, 17% of which (1 of 6) gave rise to pre-B-cell colonies. Part of the study (FL + IL-6, FL + IL-11) was duplicated on two different dates. Myeloid potential was tested under two different conditions of secondary myeloid suspension culture, one containing IL-3, PWM-SCM, and Epo and the other containing FL, Epo, and either IL-6, IL-11, or G-CSF, depending on which factor was used in the primary culture. Small samples of each culture were procured every 2 or 3 days for serial cytologic assessment of the myeloid potentials of the progenitors. Except for four cells that did not yield sufficient number of cells for the analyses, all cells that gave rise to primary colonies showed differentiation along myeloid lineages (data not shown). These results confirmed the direct effects of FL in support of the clonal proliferation of primitive lymphohematopoietic progenitors.

Inhibitory effects of IL-3 and IL-1α on the B-lymphoid potential of FL-induced colonies

<table>
<thead>
<tr>
<th>Test Cytokine in Primary Culture</th>
<th>No. of Primary Colonies/Dish</th>
<th>Mean Cell No. of Pre-B-Cell Colonies (×10^-3)</th>
<th>Average No. of Pre-B-Cell Colonies/Primary Colonies</th>
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</thead>
<tbody>
<tr>
<td>FL, IL-11</td>
<td>12 ± 2</td>
<td>1.2</td>
<td>152 ± 3</td>
</tr>
<tr>
<td>FL, IL-11, IL-3</td>
<td>67 ± 1</td>
<td>3.2</td>
<td>0</td>
</tr>
<tr>
<td>FL, IL-11, IL-1α</td>
<td>77 ± 3</td>
<td>0.15</td>
<td>0</td>
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<td>FL, IL-6</td>
<td>21 ± 3</td>
<td>2.2</td>
<td>27 ± 4</td>
</tr>
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<td>FL, IL-6, IL-3</td>
<td>63 ± 3</td>
<td>4.0</td>
<td>0</td>
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<td>FL, IL-6, IL-1α</td>
<td>24 ± 3</td>
<td>0.67</td>
<td>0</td>
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<tr>
<td>FL, G-CSF</td>
<td>10 ± 2</td>
<td>0.07</td>
<td>94 ± 0</td>
</tr>
<tr>
<td>FL, G-CSF, IL-3</td>
<td>51 ± 4</td>
<td>0.60</td>
<td>0</td>
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<tr>
<td>FL, G-CSF, IL-1α</td>
<td>18 ± 1</td>
<td>0.02</td>
<td>1 ± 1</td>
</tr>
</tbody>
</table>

One hundred fifty Lin- Ly-6A/E+ post-5-FU marrow cells were cultured in the presence of designated factors. On day 10 of the culture, colony number was scored, and 30 colonies were picked and washed. Aliquots of 1/60 of the pooled cells were plated in secondary culture containing SF and IL-7. The number of pre-B-cell colonies was counted on day 11 of secondary culture. Purified IL-3 was used in this experiment.
Fig 1. B220 expression by M'T-E- cells of normal marrow. B220- and B220+ cells were clearly separable.

with that of SF. We prepared two sets of B-cell progenitor populations from normal bone marrow. M'T-E marrow cells were sorted based on B220 expression (Fig 1). Ten thousand M'T-E B220- cells and M'T-E B220+ cells were cultured in suspension with growth factors for 11 days, and the number of B220+ cells were analyzed by flow cytometry. The results presented in Fig 2 are representative of three experiments. The detection threshold of the B220+ cells after 11 days of culture was 3 x 10^3 cells. IL-7 alone maintained the survival of B220+ cells. However, IL-7 did not support production of B220+ cells from M'T-E B220- marrow cells. SF alone did not support survival or production of B220+ cells from either population. However, in contrast with SF, FL alone supported the production of significant number of B220+ cells from M'T-E B220- marrow cells. Although the number of the B220+ cells was only 5-times more than the detection threshold and about 100-times less than that supported by IL-7 and SF, this effect of FL was shown repeatedly and only with M'T-E B220- cells. FL acted synergistically with IL-7 giving rise to B220+ cells from both M'T-E B220- and M'T-E B220+ marrow cells. FL synergized with SF in production of B220+ cells from M'T-E B220- marrow cells, but not from M'T-E B220+ marrow cells. Addition of FL to the combination of SF and IL-7 enhanced 3- to 5-fold B220+ cell production from both populations of cells.

DISCUSSION

The presence of flt3/flk2 tyrosine kinase receptor on murine fetal liver primitive hematopoietic progenitors, human bone marrow primitive hematopoietic progenitors, murine immature thymocytes suggests that the ligands for the receptor is important in hematopoiesis as well as in lymphopoiesis. Recently, both murine and human FL have been cloned and proven to stimulate the proliferation of hematopoietic progenitor cells and fetal thymocytes. In our study, FL plus IL-6, IL-11, or G-CSF supported the proliferation of primitive hematopoietic progenitors including lymphohematopoietic progenitors that are capable of differentiation along both myeloid and B-lymphoid lineages. Earlier, we observed that both IL-3 and SF act synergistically with IL-6, G-CSF, IL-11, or IL-12 in support of colony formation from dormant murine hematopoietic progenitors, and that SF-, but not IL-3-containing two-factor combinations, support B-cell potential of lymphohematopoietic progenitors. In this regard, FL was similar to SF, although the number and size of the colonies supported by FL-containing factor combinations were smaller than those supported by SF-containing factor combinations and FL did not synergize with IL-12. FL, like SF, synergized with IL-7 in support of proliferation of B+ progenitors. These functional redundancies between SF and FL might be explained by their common enzymatic role as a tyrosine kinase, and SF and FL might share common signal transduction pathways.

However, there were differences between FL and SF in their effects on both lymphohematopoietic progenitors and B-cell progenitors. The progeny of Lin- Ly-6A/E+ progenitors remained blastic much longer than the colonies supported by respective SF-containing cytokine combinations, especially in cultures containing FL plus IL-11. This may indicate only time-course differences or may signify more fundamental differences in the effects of the two cytokines on the differentiation of the progenitors. Studies are under way to clarify the mechanisms of apparent differences in the effects of FL and SF. Second, FL by itself supported the proliferation of B-cell progenitors and their maturation to B220+ cells, whereas SF alone did not. FL synergized with SF in support of the proliferation of B-cell progenitors. In this context, FL behaved like a B-cell factor. However, the targets of FL may be at earlier stages of B-lymphopoiesis than those of IL-7, because FL and FL plus SF supported cell proliferation only from M'T-E B220- progenitors, but not from B220+ progenitors. In contrast, IL-7 showed effects on M'T-E B220+ progenitors and the synergistic interaction between IL-7 and SF was observed with both B220- and B220+ progenitors.

Fig 2. Generation of B220+ cells in suspension culture from M'T-E B220- and B220+ cells. Sorted cells (1 x 10^5) were cultured with designated growth factors in a 24-well plate. On day 11 of incubation, cells were procured and analyzed for B220 expression. Purified recombinant SF was used in this experiment.
B220 progenitors. Recently, Lemischka et al (manuscript in preparation) observed relative reduction in pro-B cells and some of the pre-B cells, but not in cells at later stages of B-lymphopoiesis in \textit{flt3/flk2} null mice. Taken together, these results may indicate a unique role of FL and its receptor in early B-lymphopoiesis.

Although we have documented the effects of FL on primitive lymphohematopoietic progenitors in culture, the significance of our observations in the physiology of early hematopoiesis is unknown. The transgenic \textit{flt3/flk2} null mice showed no apparent defects in myelopoiesis. The overlapping in vitro effects of FL and SF indicate physiologic redundancy in the cytokine regulation of early hematopoiesis. In the fetal liver of \textit{Slk}/Slk mice there are significant number of hematopoietic progenitors,\textsuperscript{53} indicating that hematopoiesis in the fetal liver is regulated by mechanisms other than SF. Because \textit{flk2} is expressed by primitive progenitors in fetal liver,\textsuperscript{5} it is conceivable that the FL-flt3/flk2 system may also be involved in the early stage of hematopoiesis at least during ontogeny.

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