gp130 and c-Kit signalings synergize for \textit{ex vivo} expansion of human primitive hemopoietic progenitor cells

( soluble receptor/signal transduction/cell proliferation )

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ABSTRACT gp130, a signal-transducing receptor component of interleukin 6 (IL-6), associates with an IL-6 and IL-6 receptor (IL-6R) complex and transduces signals. To examine the role of gp130 signaling in the expansion of human hemopoietic progenitor cells, we tested the effects of a recombinant soluble human IL-6 receptor (sIL-6R) and/or IL-6 in combination with other cytokines on purified human umbilical cord blood CD34+ cells, using methylcellulose clonal assay and suspension culture in the presence or absence of serum. A combination of sIL-6R and IL-6 (sIL-6R/IL-6), but not sIL-6R or IL-6 alone, was found to dramatically stimulate expansion of hemopoietic progenitor cells as well as CD34+ cells in the presence of stem cell factor. Significant generation of multipotent hemopoietic progenitors over a period of 3 weeks in suspension culture and efficient formation of colonies, especially multilineage and blast cell colonies, in methylcellulose assay supplemented with a combination of sIL-6R/IL-6 together with stem cell factor were observed in serum-containing and serum-free culture. Addition of anti-gp130 monoclonal antibodies or anti-IL-6R monoclonal antibodies to the above cultures dose-dependently inhibited the expansion of progenitor cells in suspension culture and also completely blocked the formation of multilineage colonies in methylcellulose culture. These findings demonstrated that the significant expansion of human primitive hemopoietic progenitors could be achieved with the gp130 and c-Kit signalings initiated by the sIL-6R/IL-6 complex in the presence of stem cell factor and suggested the possible application of this method for \textit{ex vivo} expansion of CD34+ cells for bone marrow transplantation.

The interleukin 6 (IL-6) receptor (IL-6R) system comprises two functionally different chains: a ligand-binding chain (IL-6R) and a non-ligand-binding but signal-transducing chain (gp130). gp130 associates with the IL-6/IL-6-R complex, resulting in the formation of high-affinity IL-6 binding sites, and transduces the signal (1-4). An extracellular soluble form of the receptor (sIL-6R) has been shown to mediate the IL-6 signal through membrane-anchored gp130. A complex of sIL-6R and IL-6 (sIL-6R/IL-6) associates with the ubiquitously expressed gp130 on IL-6R-negative and -positive cells to induce homodimerization of gp130 and activation of the JAK-STAT pathway leading to cellular response (4-8). IL-6 has been shown to act synergistically with IL-3 and stem cell factor (SCF) to augment proliferation of human hemopoietic progenitor cells and support colony formation from dormant murine hemopoietic progenitors (9-11). However, little is known about the role of the gp130 signaling pathway in human hemopoiesis.

There has recently been great interest in the \textit{ex vivo} expansion of hemopoietic progenitor cells for a variety of clinical applications, such as the augmentation or supplantation of bone marrow transplantation and gene therapy. Although expansion of large cell numbers has often been obtained in a number of previous reports using various combinations of cytokines or stromal cells, the magnitude of progenitor cells, especially multipotential progenitors, has been usually low, suggesting that differentiation, accompanied by depletion of primitive cells, occurs in cultures (12-15). Recent study has demonstrated that a combination of sIL-6R/IL-6 sustains self-renewal of pluripotential embryonic stem cells through the activation of the gp130 signaling process (16). Thus it is interesting to investigate the potential role of gp130 signaling, which can be initiated by sIL-6R/IL-6 on human hemopoietic stem/progenitor cells.

The results presented here indicate that gp130 signaling in the presence of SCF dramatically stimulates expansion of human primitive hemopoietic progenitor cells \textit{in vitro}.

MATERIALS AND METHODS

Cell Preparation. Human umbilical cord blood, collected according to institutional guidelines, was obtained during normal full-term deliveries. Mononuclear cells (MNCs) were separated by Ficoll/Hypaque density gradient centrifugation after depletion of phagocytes with silica (IBL, Fujioka, Japan). CD34+ cells were purified from MNCs by using Dynabeads M-450 CD34 and DETACHaBEAD CD34 (Dynal, Oslo). Eighty-five to 95% of the cells separated were CD34+ by fluorescence-activated cell sorting (Ortho Diagnostics) analysis.

Receptor and Cytokines. Recombinant human IL-6 and sIL-6R were prepared as described (17, 18). Recombinant human SCF was supplied by Amgen Biologicals. Recombinant human IL-3, granulocyte/macrophage colony-stimulating factor (GM-CSF), and erythropoietin (EPO) were generously provided by Kirin Brewery (Tokyo). Recombinant human granulocyte colony-stimulating factor (G-CSF) was kindly provided by Chugai Pharmaceutical (Tokyo). All cytokines were pure recombinant molecules and were used at concentrations that induced optimal response in methylcellulose culture of human hemopoietic cells. These concentrations are

Abbreviations: IL, interleukin; IL-6R, IL-6 receptor; sIL-6R, soluble IL-6R; SCF, stem cell factor; mAb, monoclonal antibody; CFU, colony-forming unit; BSA, bovine serum albumin; FBS, fetal bovine serum; EPO, erythropoietin; GM-CSF, granulocyte/macrophage colonystimulating factor; G-CSF, granulocyte CSF; GEMM, granulocyte/erythrocyte/macrophage/megakaryocyte.

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100 ng of SCF per ml, 200 units of IL-3 per ml, 2 units of EPO per ml, and 10 ng of G-CSF and GM-CSF per ml.

**Preparation of Antibodies.** Preparation of anti-human gp130 monoclonal antibodies (mAbs) (GPX7, GPX22, and GPZ35) has been described (2, 19). The three mAbs recognize different epitopes on gp130 and were shown to inhibit IL-6-mediated biological response through inhibition of the IL-6-induced association of gp130 and IL-6 receptors. Anti-human IL-6R (PM1) mAb was prepared as described (20). PM1 was shown to inhibit IL-6-mediated biological response through inhibition of the binding of IL-6R to IL-6.

**Suspension Culture.** Purified CD34+ cells were incubated in suspension culture using a modification of previously described techniques (21, 22). One milliliter of mixture containing 2000 CD34+ cells, α-medium (Flow Laboratories), 20% fetal bovine serum (FBS; HyClone), 1% crystalized and deionized fraction V bovine serum albumin (BSA; Sigma), and different combinations of cytokines was incubated in 24-well tissue plates (Nunc) at 37°C in a humidified atmosphere flushed with 5% CO2/5% O2/90% N2. Serum-free suspension culture consisted of 2% pure BSA (Sigma), 10 μg of insulin per ml, 200 μg of transferrin per ml (Sigma), 0.01 mM 2-mercaptoethanol (Eastman), and 40 μg of low-density lipoprotein per ml (Sigma), instead of FBS and BSA (22). At weekly intervals, cultures were demp-depopulated by removal of half the culture volume, which was then replaced by newly prepared medium with the same combinations of cytokines. Cells in the collected media were washed and counted. Total hematopoietic progenitor cells generated at each time point in the culture were evaluated by culturing a fraction of expanded cells in the clonal methylcellulose assay as described below. For blocking studies, anti-gp130 or IL-6R mAbs were added at the beginning of the culture.

**Clonal Culture.** The inoculated CD34+ cells and their progenies in the suspension culture were incubated in triplicate at concentrations of 500 cells per ml for CD34+ cells and 2–10 × 103 cells per ml for cultured cells in methylcellulose culture as previously reported (23). One milliliter of culture mixture containing cells, α-medium, 0.9% methylcellulose (Shinetsu Chemical, Tokyo), 30% FBS, 1% BSA, 0.05 mM 2-mercaptoethanol, and various combinations of cytokines with or without sIL-6R was plated in each 35-mm Lux standard non-tissue culture dish and incubated at 37°C in a humidified atmosphere flushed with 5% CO2 in air. Serum-free methylcellulose culture contained components identical to those in serum-containing culture except 1% pure BSA, 300 μg of human transferrin per ml, 160 μg of soybean lecithin per ml (Sigma), and 96 μg of cholesterol per ml (Nacalai Tesque, Kyoto) replaced BSA and FBS (11). A combination of SCF, IL-3, IL-6, EPO, and G-CSF was used for the determination of various progenitors generated in suspension culture at each time point. All cultures were done in triplicate and scored at day 14 according to the criteria as reported previously (10, 11, 23, 24). The abbreviations used for the colony types are as follows: GM, granulocyte/macrophage colonies; Meg, megakaryocyte colonies; B, erythroid bursts; Blast, blast cell colonies; Mix, mixed hematopoietic colonies; and GEMM, granulocyte/erythrocyte/macrophage/megakaryocyte colonies.

**RESULTS**

**Effect of sIL-6R, IL-6, and SCF on Colony Formation from CD34+ Cells in Methylcellulose Culture.** When normal human CD34+ cells isolated from cord blood were cultured with sIL-6R and IL-6 in the presence of SCF, a significant increase in the number of cells, including numerous erythroid cells, was observed, where sIL-6R at 1280 ng/ml, IL-6 at 50 ng/ml, and SCF at 100 ng/ml were optimal combinations for the increase in the culture (unpublished data). This observation suggested a possibility that the sIL-6R/IL-6 complex in the presence of SCF may stimulate proliferation of hematopoietic progenitors, resulting in the increase of cells in the culture. To examine this possibility, we first carried out methylcellulose clonal culture of CD34+ cells with various cytokines in combinations with 1280 ng of sIL-6R per ml, 50 ng of IL-6 per ml, and/or 100 ng of SCF per ml (Table 1). In serum-containing culture, sIL-6R, IL-6, sIL-6R/IL-6, or SCF alone induced only a small number of colonies. A combination of IL-6 and SCF enhanced the formation of GM and Blast colonies compared with IL-6 or SCF alone. A most striking generation of colonies was observed in the culture supplemented with sIL-6R, IL-6, and SCF at a plating efficiency as high as >50%. Addition of sIL-6R to the combination of IL-6 and SCF increased total colony numbers 4.2-fold, and the number of colonies induced by the three factors was 11.3-fold, 5.2-fold, and 5.4-fold compared with that of colonies by IL-3, GM-CSF, and G-CSF, respectively. Interestingly, considerable numbers of Mix colonies with large size, most of which were GEMM, and Blast colonies were developed in addition to a number of Meg colonies and erythroid bursts in culture with sIL-6R, IL-6, and SCF. More than 60% of the colonies induced by the three factors were GEMM and Blast colonies, whereas most of the colonies induced by IL-3, GM-CSF, and G-CSF were GM colonies.

To exclude the possible influences of some unknown factor(s) in FBS, serum-free culture was carried out. A most significant colony formation was again observed in the culture with IL-6, sIL-6R, and SCF. The addition of sIL-6R to the combination of IL-6 and SCF increased total colony numbers 17.5-fold and stimulated the formation of a large number of Mix and Blast colonies in addition to Meg colonies and erythroid bursts, whereas no colonies or only a few GM colonies developed in other factor combinations.

When sIL-6R/IL-6 was tested in combination with other factors as shown in Table 1, slight synergy between sIL-6R/IL-6 and either IL-3, GM-CSF, or G-CSF was observed in serum-containing culture. However, no synergy was found between sIL-6R/IL-6 and these factors in serum-free culture. This result indicates that sIL-6R/IL-6 specifically synergizes with SCF for the proliferation of CD34+ progenitor cells.

**Effect of sIL-6R, IL-6, and SCF on Expansion of Hematopoietic Progenitor Cells in Suspension Culture.** The results shown in methylcellulose culture suggest that sIL-6R/IL-6 in combination with SCF may be useful for the expansion of hematopoietic progenitor cells. We next carried out serum-containing suspension culture of CD34+ cells with SCF alone or in combination with IL-6 in the presence and absence of various concentrations of sIL-6R. Progenitors derived from the culture were assayed after 14 days of incubation. As shown in Fig. 1A, total progenitors dramatically increased in accordance with the concentration of sIL-6R. This increase was detectable with sIL-6R at a concentration as low as 80 ng/ml and reached a plateau at 1280 ng/ml with about a 70-fold increase of progenitors. In the absence of IL-6, however, sIL-6R failed to expand the total progenitor cells. The expansion of progenitors also depended on the concentration of IL-6, and the maximal fold increase of progenitors was obtained at concentrations exceeding 50 ng/ml in the presence of sIL-6R (Fig. 1B). In contrast, in the absence of sIL-6R, only about a 10-fold increase of progenitors by IL-6 with SCF was observed even at concentrations over 50 ng/ml. These results indicate that sIL-6R is functional and capable of transducing proliferative signals in CD34+ cells only in combination with IL-6, and 1280 ng of sIL-6R per ml and 50 ng of IL-6 per ml were the optimal concentrations in the presence of SCF for the expansion of progenitors in suspension culture.

To examine the effect of sIL-6R/IL-6 on expansion of hematopoietic progenitor cells in more detail, serum-containing and serum-free suspension cultures supplemented with sIL-6R and IL-6 in combination with other factors over a period of 3 weeks were carried out with weekly analysis of progenitor cells. Fig. 2 shows the results from serum-containing suspension
Table 1. Colony formation from CD34+ cells of human umbilical cord blood in methylcellulose culture

<table>
<thead>
<tr>
<th>Factor(s)</th>
<th>GM</th>
<th>Blast</th>
<th>Meg</th>
<th>B</th>
<th>Mix</th>
<th>Total</th>
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<td></td>
<td></td>
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<tr>
<td>IL-6</td>
<td>6 ± 0.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6 ± 0.6</td>
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<tr>
<td>sIL-6R</td>
<td>3 ± 0.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 ± 0.7</td>
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<tr>
<td>IL-6 + sIL-6R</td>
<td>13 ± 0.7</td>
<td>1 ± 0.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14 ± 1.9</td>
</tr>
<tr>
<td>SCF</td>
<td>13 ± 2.5</td>
<td>1 ± 0.7</td>
<td>1 ± 0.7</td>
<td>0</td>
<td>0</td>
<td>15 ± 2.1</td>
</tr>
<tr>
<td>+ IL-6</td>
<td>54 ± 4.3</td>
<td>10 ± 3.1</td>
<td>1 ± 0.6</td>
<td>0</td>
<td>0</td>
<td>65 ± 7.4</td>
</tr>
<tr>
<td>+ IL-6 + sIL-6R</td>
<td>62 ± 3.7</td>
<td>45 ± 7.2</td>
<td>13 ± 2.6</td>
<td>28 ± 5.8</td>
<td>122 ± 22.8</td>
<td>270 ± 17</td>
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<tr>
<td>IL-3</td>
<td>23 ± 1.8</td>
<td>1 ± 0.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>24 ± 1.7</td>
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<tr>
<td>+ IL-6 + sIL-6R</td>
<td>29 ± 2.1</td>
<td>12 ± 5.0</td>
<td>1 ± 0.7</td>
<td>30 ± 4.9</td>
<td>21 ± 7.1</td>
<td>93 ± 7.1</td>
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<td>0</td>
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<td>52 ± 10</td>
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<tr>
<td>+ IL-6 + sIL-6R</td>
<td>89 ± 6.8</td>
<td>8 ± 3.1</td>
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<td>0</td>
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<td>97 ± 11</td>
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<td>G-CSF</td>
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<td>50 ± 6.6</td>
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<tr>
<td>+ IL-6 + sIL-6R</td>
<td>61 ± 4.0</td>
<td>6 ± 3.4</td>
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<td>67 ± 7.8</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>+ sIL-6R</td>
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<tr>
<td>SCF</td>
<td>1 ± 1.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 ± 1.7</td>
</tr>
<tr>
<td>+ IL-6</td>
<td>4 ± 0.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4 ± 2.1</td>
</tr>
<tr>
<td>+ IL-6 + sIL-6R</td>
<td>5 ± 1.8</td>
<td>7 ± 3.3</td>
<td>5 ± 2.1</td>
<td>8 ± 3.5</td>
<td>45 ± 4.3</td>
<td>70 ± 5.4</td>
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<td>5 ± 0.7</td>
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<td>5 ± 0.7</td>
</tr>
<tr>
<td>+ IL-6 + sIL-6R</td>
<td>6 ± 1.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6 ± 1.9</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>1 ± 0.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 ± 0.5</td>
</tr>
<tr>
<td>+ IL-6 + sIL-6R</td>
<td>3 ± 1.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 ± 1.0</td>
</tr>
<tr>
<td>G-CSF</td>
<td>3 ± 0.8</td>
<td>0</td>
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<td>0</td>
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<td>3 ± 0.8</td>
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<tr>
<td>+ IL-6 + sIL-6R</td>
<td>3 ± 1.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>3 ± 1.4</td>
</tr>
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</table>

Cells were cultured in the presence of designated factor combinations and colonies were scored on day 14. The number of colonies indicates mean ± SD of triplicate cultures. See text for abbreviations.

When compared with the preexpansion value, the overall increase in the progenitors was 44-fold, 61-fold, and 33-fold by days 7, 14, and 21, respectively. About an 80-fold increase of CD34+ cells by fluorescence-activated cell sorting analysis at day 14 of culture was also observed.

Weekly analyses of different subtypes of expanded progenitors in methylcellulose assay showed that all types of progenitors including GM colony-forming units (CFU-GM), erythroid burst-forming units (BFU-E), CFU-Blast and CFU-Mix continued to be generated throughout 3 weeks of culture in the presence of sIL-6R, IL-6, and SCF, although Mix colonies were

![Fig. 1](image1.png)

**Fig. 1.** Expansion of progenitor cells by sIL-6R and IL-6 on 2000 human CD34+ cells containing 840 progenitors in the presence of SCF after 14 days in serum-containing suspension culture. (A) Fold increase of progenitor cells at various concentrations of sIL-6R in the presence (open circles) or absence (filled circles) of 500 ng of IL-6 per ml. (B) Fold increase of progenitor cells at various concentrations of IL-6 in the presence (open circles) or absence (filled circles) of 1280 ng of sIL-6R per ml.

![Fig. 2](image2.png)

**Fig. 2.** Generation of total progenitors from 2000 CD34+ cells containing 684 progenitors in serum-containing suspension culture supplemented with single factors or in combinations at day 7 (open bars), day 14 (oblique bars), and day 21 (filled bars). The data are presented from a single experiment. Similar data were obtained in four additional experiments.
barely detectable in other factor combinations. The number of CFU-Mix increased approximately 60-fold and 80-fold by days 7 and 14, respectively. Similar results were also obtained from serum-free suspension culture. Interestingly, considerable numbers of CFU-Mix with a 40-fold increase were obtained even at day 21 of serum-free suspension culture in the presence of sIL-6R, IL-6, and SCF.

The above results revealed that sIL-6R/IL-6 acts synergistically with SCF in expansion of progenitors. In subsequent experiments, we tested sIL-6R/IL-6 in combination with some early-acting cytokines, including IL-3 and G-CSF in the presence of SCF. We also compared the effect of the combination of sIL-6R, IL-6, and SCF with that of the combination of IL-6, IL-3, and SCF, which have been shown to be potent and extensively used for the expansion study. Expansion of total progenitors by the combination of sIL-6R, IL-6, and SCF was 1.5-fold of that by the combination of IL-6, IL-3, and SCF. Generation of CFU-Mix with different cytokine combinations in serum-containing and serum-free cultures was shown in Fig. 3. A combination of sIL-6R, IL-6, and SCF expanded CFU-Mix approximately 60-fold and 80-fold in serum-containing culture and 49-fold and 68-fold in serum-free culture by days 7 and 14, respectively. Progenitors generated by a combination of IL-6, IL-3, and SCF were mainly granulocyte and/or macrophage lineage, and CFU-Mix were only expanded about 30-fold in serum-containing and 10-fold in serum-free culture by day 14 of culture. Addition of IL-3 to the combination of sIL-6R, IL-6, and SCF did not increase the expansion of CFU-Mix, and, intriguingly, addition of G-CSF to the combination appeared to have negative effects on the expansion. The results revealed that a combination of sIL-6R, IL-6, and SCF is more potent especially on expansion of primitive progenitors.

Effects of Anti-gp130 mAbs and Anti-IL-6R mAb on the Expansion of Progenitor Cells. To verify the involvement of gp130 in the sIL-6R/IL-6 complex-mediated hematopoietic progenitor cell expansion, we examined the effects of mouse anti-human gp130 mAbs and anti-human IL-6R mAb on progenitor cell expansion. Addition of anti-gp130 mAbs dose-dependently inhibited the expansion of total progenitor cells in the serum-containing suspension culture (Fig. 4A). Expansion of CFU-Mix was completely blocked at a concentration of 1 \( \mu g/mL \), whereas the mAbs had no effect on the expansion induced by SCF and IL-3. Addition of anti-IL-6R mAb to the culture resulted in a similar fashion of inhibition except at a slightly lower efficiency, and complete abrogation of CFU-Mix expansion was observed at a concentration of 10 \( \mu g/mL \) (Fig. 4B). The anti-IL-6R also did not show any effect on expansion stimulated by SCF and IL-3. In contrast, an anti-EPO antibody inhibited the expansion induced by SCF and EPO but had no effect on that induced by sIL-6R, IL-6, and SCF (data not shown). The same results were also obtained from serum-free suspension culture as well as methylcellulose culture.

**DISCUSSION**

In this study we demonstrate that two signal pathways through gp130 and c-Kit, which are initiated by sIL-6R/IL-6 and SCF, respectively, synergistically promote potent expansion of human hematopoietic progenitor cells in suspension and dramatically enhance the formation of colonies in methylcellulose culture. sIL-6R has been reported to potentiate agonistic effects in the presence of IL-6 on some cell lines such as BAF-m130, gp130 cDNA-transfected cells, and the murine osteoclasts (6–8, 25). Here we show that sIL-6R/IL-6 in the presence of SCF is a very potent stimulator for the proliferation of human primitive hematopoietic progenitors. To our knowledge, no other cytokine has been reported to have so striking synergy with SCF in the stimulation of human primitive hematopoietic cells.

In *vitro* expansion of hemopoietic progenitor cells is an attractive way to prepare suitable hematopoietic cells for potential clinical application, including gene therapy. Previously, various combinations of cytokines were reported for the expansion of progenitor cells (12–15). SCF, IL-3, and IL-6 have been accepted as the cytokines acting on primitive hemopoietic cells, and a combination of the three was supposed to be the basic and potent combination and has been widely used in the expansion of progenitors. In accordance with this, our results also indicate the combination of SCF, IL-3, and IL-6 is potent on expansion. However, as our results demonstrated, a combination of sIL-6R with IL-6 and SCF is superior in terms of expansion rate of primitive progenitors such as CFU-Mix or CFU-Blast. Several studies based on human peripheral blood CD34+ cells showed a combination of
IL-6, IL-3, and SCF with some other cytokines such as G-CSF, GM-CSF, IL-1, or EPO was potent for the generation of progenitors in serum-containing suspension culture (12, 13). About a 60-fold increase of CFU-GM was achieved; however, CFU-Mix failed to be expanded or even no CFU-Mix or BFU-E was detected at day 14 of culture, suggesting relative late-stage progenitors were predominantly expanded in these cultures. The efficient expansion of multipotent hematopoietic progenitors in suspension culture and dramatic formation of Mix and Blast colonies in methylcellulose culture by sIL-6R, IL-6, and SCF suggest that sIL-6R/IL-6 may act on an earlier stage of primitive hematopoietic cells or even pluripotent stem cells to stimulate them to expand. Further studies will be needed to show the long-term repopulating capability of the expanded progenitors in vivo.

Significant inhibition or even complete abrogation by anti-gp130 mAbs and anti-IL-6R mAb on progenitor expansion and colony formation clearly demonstrated that the observed effects of sIL-6R/IL-6 were provided through interaction of the IL-6-bound sIL-6R molecule to membrane-anchored gp130 on the target cells. Recent studies have shown that gp130 was ubiquitously expressed on cells, and self-renewal of embryonic stem cells can be maintained by the sIL-6R/IL-6 complex without leukemia inhibitory factor in vitro (6, 16, 18). However, information remains incomplete on what cytokine receptors are normally expressed on human CD34+ hematopoietic stem/progenitor cells. Certainly, c-Kit for SCF is present and gp130 appears to be present in all CD34+ cells (unpublished data), while IL-6R appears to be present only in a small population of CD34+ cells by immunostaining studies. A complex of sIL-6R/IL-6 may enhance the IL-6 signal in IL-6R-positive cells and also, more importantly, mediate the signal via gp130 in IL-6R-negative CD34+ cells, which are normally unresponsive to IL-6. Since sIL-6R/IL-6 functions only in the presence of SCF, as revealed in the present study, coexpression of gp130 and c-Kit on progenitor cells and coactivation of the signal pathways of gp130 and c-Kit may lead to dramatic proliferation of human hematopoietic progenitor cells. The present study may provide a further approach to expand human hematopoietic stem/progenitor cells for potential clinical application.

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