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The *In Vitro* Effects of Stem Cell Factor and PIXY321 on Myeloid Progenitor Formation (CFU-GM) from Immunomagnetic Separated CD34$^+$ Cord Blood

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ABSTRACT. Two novel cytokines, stem cell factor (SCF) and PIXY321 (a fusion protein, granulocyte macrophage colony-stimulating factor + IL-3), have recently been demonstrated to enhance *in vitro* adult myelopoiesis. In this study, we compared the success of separating very early hematopoietic progenitor cells (CD34$^+$) from both cord blood (CB) and adult bone marrow (ABM) and their differential response to SCF, PIXY321, and other later-acting colony-stimulating factors (CSF). Briefly, CD34$^+$ cells were isolated from CB and ABM with an anti-CD34 MAb, HPCA-1, and incubated with various combinations of SCF, PIXY321, and other CSF. The percentage of CD34$^+$ cells was decreased in CB compared to ABM before separation (0.54 *versus* 1.71%) ($p = 0.05$). Isolated CD34$^+$ cells from CB and ABM were similar in lineage with respect to CD38, HLA-DR, CD33, and CD5, but decreased in CB with respect to B-lineage expression (CD19, CD10, and CD22) ($p = 0.05$). SCF increased colony forming unit–granulocyte-macrophage (CFU-GM) formation from CB CD34$^+$ cells compared to unconditioned media and had a significant additive increase with IL-3 ($p = 0.006$) and granulocyte colony-stimulating factor ($p = 0.03$). SCF also had an additive increase in CB CFU-GM formation with PIXY321 ($p = 0.007$). PIXY321 had a similar increase in CFU-GM formation from both CB and ABM CD34$^+$ cells compared to the combination granulocyte macrophage colony-stimulating factor + IL-3. When SCF was added to IL-3, PIXY321, or PIXY321 + IL-6, there was an increase in CFU-GM from CB *versus* ABM CD34$^+$ cells. The combination of SCF + IL-3 + IL-6 induced the highest increase in CFU-GM formation from CB CD34$^+$ cells. These data suggest that isolated CD34$^+$ cells in CB are significantly enhanced in response to early-acting CSF (SCF), especially when used in combination with late-acting CSF. These results may have implications in the *ex vivo* expansion of CB progenitor cells and the modulation of neonatal hematopoiesis. (*Pediatr Res* 32: 277–281, 1992)

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Abbreviations

SCF, stem cell factor
CSF, colony-stimulating factor
PIXY321, GM-CSF + IL-3 fusion protein
CFU-GM, colony forming unit–granulocyte-macrophage
CFU-GEMM, colony forming unit–granulocyte, erythroid, monocyte, megakaryocyte
GM-CSF, granulocyte macrophage colony-stimulating factor
G-CSF, granulocyte colony-stimulating factor
PE, phycoerythrin
rh, recombinant human
MGF, mast cell growth factor
BFU-E, burst forming unit-erythroid

The recent success of using umbilical cord blood from an HLA-matched sibling as a source of hematopoietic reconstitution in a patient with Fanconi's anemia has renewed a great interest in the understanding of neonatal hematopoiesis (1). Neonatal animal studies have suggested that the total body myeloid committed progenitor cells (CFU-GM) pool per g body weight is significantly decreased despite a high CFU-GM proliferative rate (2–5). Although total body pools (liver + spleen + bone marrow + circulating pool) of both pluripotent and unipotent myeloid progenitor cells (CFU-GEMM and CFU-GM, respectively) have not been determined in human neonates, recent human studies have suggested that the peripheral blood populations of both of these pools are significantly elevated compared to those in adults. The circulating CFU-GEMM pool in term infants is almost 3.5-fold higher and in preterm infants is 5-fold higher than similar concentrations found in adult peripheral blood (6). Similarly, the circulating CFU-GM pool in term newborns is almost 18-fold higher and in preterm newborns is 40-fold higher compared to similar concentrations found in adult peripheral blood (7). Lastly, stem-cell-type progenitor cells, which have the ability to undergo self-renewal as well as to develop into multipotential and unipotential progenitor cell colonies, have been consistently demonstrated in human umbilical cord blood but have not been quantitatively compared with levels from adult bone marrow (8–10).

During hematopoiesis, colony-forming cells mature at different stages and can be identified by their ability to form either multipotential or unipotential hematopoietic lineages. The expression of a surface membrane phosphoglycoprotein (CD34

antigen, 115 kd) on human progenitor cells has been used to help differentiate multipotential hematopoietic colony-forming cells from more mature peripheral blood elements (11, 12). CD34 expression has been demonstrated on multipotential hematopoietic progenitor cells, including CFU-blasts, CFU-GEMM, and bipotential and unipotential colony-forming cells, including CFU-GM, colony forming unit megakarocyte, BFU-E, and early B-lymphoid progenitor cells but not more mature lineages (13–15). $CD34^+$ hematopoietic cells can be identified by one of several murine MAb, including My10, BI3C5, 12.8, and ICH3 (11, 16–18). $CD34^+$ hematopoietic stem cells, however, are only present in 1–4% of Ficoll-Hypaque-separated adult bone marrow mononuclear cells but are rarely detected in adult peripheral blood (11, 15, 18). Despite their low concentrations, isolated $CD34^+$ populations of hematopoietic bone marrow progenitor cells have been used to reconstitute hematopoiesis during bone marrow transplantation of lethally irradiated baboons and in patients with neuroblastoma and breast cancer (19, 20).

Several new hematopoietic growth factors have recently been identified to stimulate hematopoiesis at a very early hematopoietic progenitor cell stage. One of these new hematopoietic growth factors, Steel factor (SCF, c-*kit* ligand, or mast cell growth factor) (21–23), has been identified to be a product of the Steel locus in the mouse and, in combination with specific late-lineage cytokines (GM-CSF, G-CSF, and IL-3), has recently been demonstrated to significantly induce an increase in CFU-GM and BFU-E colonies from isolated adult human bone marrow $CD34^+$ cells (24–27). Additionally, a novel fusion protein, PIXY321 (GM-CSF/IL-3), was recently engineered and expressed (28). PIXY321 has been demonstrated to enhance CSF receptor binding affinity and proliferative activity and significantly induce CFU-GEMM, BFU-E, and CFU-GM from adult human bone marrow cells compared to GM-CSF, IL-3, or combined GM-CSF + IL-3 (28, 29). There is little information, however, regarding the capacity of isolated $CD34^+$ progenitor cells from circulating umbilical cord blood to respond to these two novel cytokines, SCF and PIXY321. This study, therefore, was designed to develop an optimal system for isolating and separating $CD34^+$ progenitor cells from umbilical cord blood and determine their potential responses to novel combinations of early- and late-acting hematopoietic growth factors.

MATERIALS AND METHODS

Isolation of cord and adult $CD34^+$ cells. Venous cord blood was drawn from the umbilical vessels of placentas of normal, full-term, nonstressed infants immediately after delivery or at scheduled cesarean section without labor. Adult bone marrow was obtained from individuals undergoing bone marrow harvest for autologous or allogeneic transplantation. Informed consent was obtained from all adult donors. Adult donors demonstrated normal peripheral blood counts and similar CFU-GM content compared to age-matched controls. Mononuclear cells from both sources were isolated using Ficoll-diatrizoate (density = 1.077 g/mL) density gradient centrifugation (Histopaque, Sigma Chemical Co., St. Louis, MO), washed, and resuspended in RPMI (Sigma) containing 1% human serum albumin (Baxter/Hyland, Glendale, CA) (RPMI/HSA). Cell concentration was determined with an electronic cell counter (Coulter, Hialeah, FL). Mononuclear cells were incubated with 0.5 μg of the anti-CD34 MAb HPCA-1 (Becton Dickinson, Mountain View, CA) per 10^6 nucleated cells for 30 min at 4°C, washed, and resuspended at 5×10^7/mL in RPMI containing 0.5% Gammagard (Baxter/Hyland). The suspension was maintained at room temperature for 15 min. Paramagnetic microspheres coated with rat antimouse IgG1 (RAM beads) (Dynal, Oslo, Norway) were then added at 1 bead/cell, and the bead/cell mixture was incubated for 60 min at 4°C in a prototype positive selection device at 1×10^7 cells/mL in RPMI/HSA (30). The device consists of a disposable chamber (60 mL) with integral fluid paths mounted on a platform with an array of permanent magnets and a motor capable of rotating the entire assembly between 1 and 10 rpm. Rosetted cells were collected by exposing the bead/cell mixture to the magnets after incubation, washed three times with RPMI, and drained into a 15-mL polypropylene tube. The beads were released from the cells by incubation with chymopapain (Discase, Baxter, Santa Ana, CA) (200 pkat/2 $\times$ 10^8 beads/mL) for 15 min at 37°C. The cells were washed once, resuspended in RPMI/1% HSA, and counted.

Flow cytometry. Bone marrow and cord blood mononuclear cells were stained with fluorochrome-conjugated MAb. $CD34^+$ cells were measured by the 8G12 antibody conjugated to FITC or PE, which binds to a chymopapain-resistant epitope of the antigen. The 8G12 antibody was produced by Baxter/Hyland under license from Becton Dickinson and conjugated with the fluorochrome at a fluorescein/protein ratio of 3.0. Leukocyte populations were defined with Leukogate (Becton Dickinson) and other antibodies used to characterize the $CD34^+$ cells, including CD33-PE (Leu-M9), CD38-PE (Leu-17), HLA-DR-PE, CD5-PE (Leu-1), CD19-PE (Leu-12), CD10-FITC (CALLA), and CD22-PE (Leu-14) (all from Becton Dickinson). Isotype controls (Simultest Control, Becton Dickinson) were included for each staining experiment. Stained cells were read with a FACScan flow cytometer and analyzed with LYSYS software (Becton-Dickinson).

Cytokines. SCF, c-*kit* ligand, or mast cell growth factor, is the product of the Steel locus in the mouse and also located on the human chromosome 12. RhSCF was produced in *Escherichia coli*, and was kindly provided as purified material by Krisztina Zsebo, Ph.D., Amgen Biologicals, Inc., Thousand Oaks, CA. PIXY321, the GM-CSF/IL-3 fusion protein, is generated by the construction of a plasmid in which the coding of the human GM-CSF and IL-3 cDNA are connected by a synthetic linker sequence followed by the subsequent expression in yeast and purification to homogeneity. PIXY321 has been shown to bind to the cell-surface receptor of either the GM-CSF or IL-3 domains. It was a generous gift from Steven Gillis, Ph.D., and Douglas Williams, Ph.D., Immunex Corporation, Seattle, WA. G-CSF (Neupogen) was also obtained from Amgen, GM-CSF (Sargramostim) from Hoechst-Roussel (Somerville, NJ), IL-3 from Gibco BRL (Grand Island, NY), and IL-6 from Genzyme (Cambridge, MA). All cytokines were used at a concentration of 100 ng/mL, which was determined to be optimal for colony culture.

Colony assays. Isolated $CD34^+$ cells were suspended in Iscove's modified Dulbecco's media with 20% modified FCS, and cultured in Iscove's modified Dulbecco's medium/0.9% methylcellulose (Terry Fox Laboratories, Vancouver, BC), 20% modified FCS, 2-mercaptoethanol, and erythropoietin (2 U/mL) (Gibco BRL). Specific cytokines or combinations of cytokines (as described above) were added to cultures to determine their effect on CFU-GM formation, and phytohemagglutinin leukocyte conditioned media (Terry Fox Laboratories) was used to assess positive nonspecific control growth. Cells were plated in 24-well plates (Costar, Cambridge, MA) at 10^2 cells per well and incubated in a high humidity incubator at 37°C, 5% CO_2. Colonies were evaluated after 14 d in culture, and clusters of 50+ cells were considered colonies.

Statistical analysis. Results are expressed as mean values ± SEM of five cord and adult $CD34^+$ isolations and triplicate wells of their cytokine/colony cultures. The probability of significant differences when comparing two groups was determined with the use of one- and two-tailed unpaired *t* tests, and the probability of significant differences when examining multiple groups was determined by using the analysis of variance followed by the Student-Newman-Keuls multiple range test to define the unique subsets within the study. Statistical analyses were performed using the Biostat I statistical program (Sigma Soft, Placentia, CA) for the IBM personal computer. p values < 0.05 are considered significant.

RESULTS

The percentage of $CD34^+$ was determined in both cord blood and adult bone marrow before immunomagnetic separation with 8G12-FITC. The percentage of $CD34^+$ cells in cord blood was $0.54 \pm 0.09\%$ compared to $1.71 \pm 0.60\%$ in adult bone marrow ($p = 0.05$) ($n = 7$). After separation, the percentage yield from cord blood was $86.7 \pm 7.48\%$ compared to $91.7 \pm 3.8\%$ in adult bone marrow, representing a percentage of recovery of $67.0 \pm 9.56\%$ from cord blood compared to $42.3 \pm 7.34\%$ from adult bone marrow (p = NS).

The immunophenotyping characteristics of the isolated $CD34^+$ population from cord blood and adult bone marrow were similar with respect to CD38 and HLA-DR expression (Table 1). Similarly, $CD34^+$ cells were myeloid and T-cell lineage negative (CD33 and CD5, respectively) from both cord blood and adult bone marrow (Table 1). However, there was a significant difference relative to B-lineage expression from the $CD34^+$ population in cord blood compared to adult bone marrow (Table 1) (CD19 cord blood $2.63 \pm 0.15\%$ *versus* adult bone marrow $36.5 \pm 7.28\%$ $p = 0.05$.) A similar change in expression in CD10 and CD22 was also demonstrated in cord blood compared to adult bone marrow $CD34^+$ progenitors (CD10, $10 \pm 0.1\%$ *versus* $33 \pm 14.9\%$, $p < 0.01$; CD22, $6.0 \pm 0.1\%$ *versus* $52.5 \pm 14.2\%$, $p < 0.01$) (cord blood *versus* adult bone marrow). We next evaluated the additive effect of incubating SCF with isolated $CD34^+$ cord blood with each of the following cytokines: IL-6, IL-3, GM-CSF, and G-CSF. SCF alone increased CFU-GM colony formation from cord blood $CD34^+$ progenitor cells compared to conditioned media and control [$21.7 \pm 2.1\%$ (SCF alone) *versus* $5.8 \pm 1.6\%$ (negative control) ($p = 0.001$) *versus* $20.0 \pm 0.17\%$ phytohemagglutinin (positive control) (p = NS)]. SCF significantly increased CFU-GM colony formation when combined with either IL-3 ($p = 0.006$), G-CSF ($p = 0.03$), or GM-CSF ($p = 0.09$) (Fig. 1). There was no difference between IL-3, G-CSF, or G-CSF when combined with SCF. However, SCF had no significant increase on cord blood CFU-GM colony formation when combined with IL-6 ($11.5 \pm 2.5\%$ *versus* $28.1 \pm 5.4\%$) (p = NS).

We additionally evaluated the effect of PIXY321 compared to combined IL-3 + GM-CSF on cord blood $CD34^+$ progenitor cell formation. There was no significant difference between PIXY321 and combined IL-3 + GM-CSF ($17.11 \pm 1.1\%$ *versus* $16.3 \pm 2.1\%$) (p = NS). Similarly, PIXY321 compared to IL-3 + GM-CSF with adult bone marrow $CD34^+$ cells induced similar CFU-GM formation ($11.2 \pm 0.7\%$ *versus* $14.3 \pm 1.4\%$) (p = NS). However, SCF induced a significantly higher increase in CFU-GM formation from $CD34^+$ cord blood when combined with PIXY321 ($p = 0.007$) (Fig. 2). Additionally, SCF induced a significant increase in CFU-GM formation when combined with IL-3 + GM-CSF compared to IL-3 + GM-CSF ($p = 0.004$) (Fig. 2). SCF also induced a higher increase in CFU-GM formation from $CD34^+$ cord blood progenitor cells with PIXY321 compared to IL-3 + GM-CSF ($50.1 \pm 8.1\%$ *versus* $38.3 \pm 4.3\%$) (Fig. 2).

We also determined the additive effect of SCF, IL-3, and SCF + IL-3 + IL-6 on cord blood CFU-GM colony formation from $CD34^+$ progenitor cells. There was no significant difference between SCF and IL-3 + IL-6 in inducing CFU-GM colony formation (Fig. 3). However, SCF + IL-3 combined with IL-6 was significantly higher than IL-3 or SCF + IL-6 and, in fact, produced the highest increase in CFU-GM colony formation from cord blood $CD34^+$ progenitor cells (Fig. 3). IL-6 + IL-3 + SCF induced a significantly higher increase in CFU-GM than did IL-3 + GM-CSF + SCF ($p < 0.05$).

In comparison, SCF induced a higher increase in CFU-GM colony formation from $CD34^+$ cord blood compared to adult bone marrow when combined with either IL-3, PIXY321, or PIXY321 + IL-6 (Fig. 4). There was, however, no significant difference when adding SCF to GM-CSF, G-CSF, IL-6, IL-3 + GM-CSF, or IL-6 + IL-3 from cord blood compared to adult

Table 1. *Immunophenotyping characteristics of $CD34^+$ cord blood and adult bone marrow*

	Cord blood (%)	Adult bone marrow (%)
$CD33^+$	1.24	0.50
$CD38^+$	88.3	98.1
HLA-DR	88.4	97.5
CD5	0.88	1.34
CD19	2.63	36.5

* $p < 0.05$

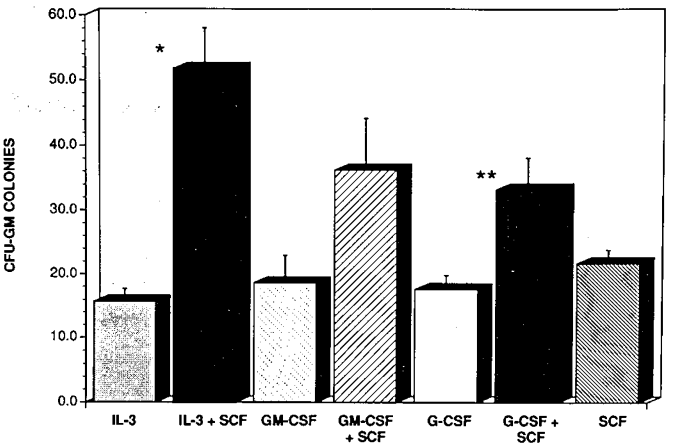

Fig. 1. The effect of IL-3, GM-CSF, and G-CSF with and without SCF on CFU-GM formation from isolated $CD34^+$ progenitors from cord blood. $CD34^+$ cells were plated at 100 cells per mL in methylcellulose culture media. Erythropoietin (1 U/mL) was added to each culture, and specific cytokines or the combination of SCF and cytokine (100 ng/mL) was added to triplicate cultures. Results reflect the mean number of colonies ± SEM of five experiments (from separate cord blood samples) scored after 14 d incubation. *, IL-3 *vs* IL-3/SCF, $p = 0.006$; **, G-CSF *vs* G-CSF + SCF, $p = 0.03$.

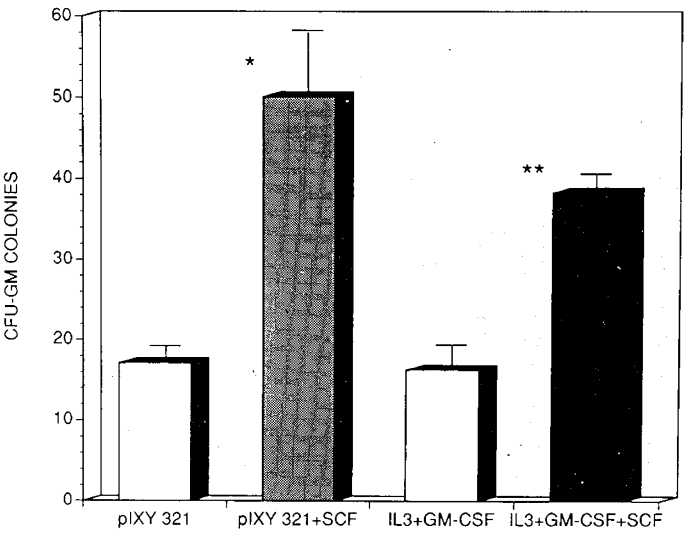

Fig. 2. The effect of PIXY321 and the combination of IL-3 + GM-CSF with and without SCF on CFU-GM formation from isolated $CD34^+$ progenitors from cord blood. $CD34^+$ cells were plated at 100 cells per mL in methylcellulose culture media. Erythropoietin (1 U/mL) was added to each culture, and specific cytokines or the combination of SCF and cytokine (100 ng/mL) was added to triplicate cultures. Results reflect the mean number of colonies ± SEM of five experiments scored after 14 d incubation. *, PIXY321 *vs* PIXY321 + SCF, $p = 0.007$; **, IL-3 + GM-CSF *vs* IL-3 + GM-CSF + SCF, $p = 0.004$.

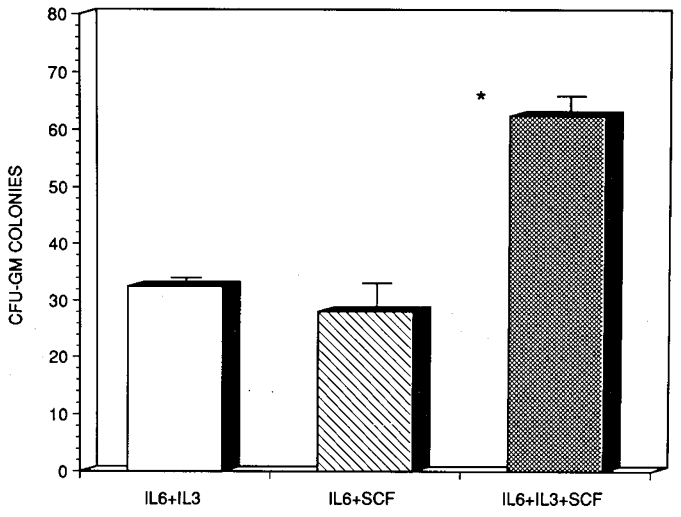

Fig. 3. The effect of IL-6 and the combination of IL-6 + IL-3 with and without SCF on CFU-GM formation from isolated CD34$^+$ progenitors from cord blood. CD34$^+$ cells were plated at 100 cells per mL in methylcellulose culture media. Erythropoietin (1 U/mL) was added to each culture, and specific cytokines or the combination of SCF and cytokine (100 ng/mL) was added to triplicate cultures. Results reflect the mean number of colonies ± SEM of five experiments scored after 14 d incubation. *, IL-6 + IL-3 *vs* IL-6 + IL-3 + SCF, $p = 0.012$.

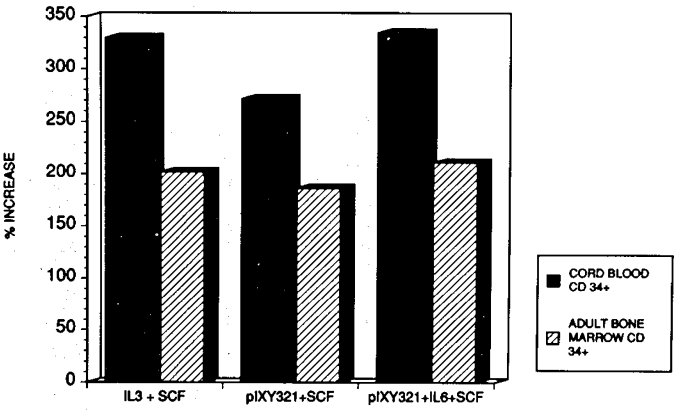

Fig. 4. The effect of IL-3, PIXY321, and the combination of PIXY321 + IL-6 with and without SCF on CFU-GM formation from isolated CD34$^+$ progenitors from cord blood and adult bone marrow. CD34$^+$ cells were plated at 100 cells per mL in methylcellulose culture media. Erythropoietin (1 U/mL) was added to each culture, and specific cytokines or the combination of SCF and cytokine (100 ng/mL) was added to triplicate sets of cultures. Five experiments were scored after 14 d incubation. Results reflect the % increase of CFU-GM colonies cultured with the single cytokine or combinations of cytokine and the colonies cultured with added SCF.

bone marrow CD34$^+$ progenitor cells. The highest increase in CFU-GM formation from adult bone marrow CD34$^+$ progenitor cells, however, occurred under the conditions of SCF + IL-3 + GM-CSF (26.5 ± 2.8%) as compared to IL-3 + GM-CSF alone (14.3 ± 1.0%) ($p = 0.05$). This was significantly less than the optimal combination of SCF + IL-3 + IL-6 with cord blood CD34$^+$ cells (62.5 ± 3.5% *versus* 26.5 ± 2.8%) (cord blood *versus* adult bone marrow) ($p = 0.01$). Lastly, there was no significant difference in CFU-GM colony size in any of the above-mentioned results.

DISCUSSION

Umbilical cord blood contains enough early and committed progenitor cells for reconstitution during allogeneic bone marrow transplantation. Cord blood has been demonstrated to increase CFU-GM colony formation from unseparated and CD34$^+$ separated cells by a number of hematopoietic growth factors, including IL-3, GM-CSF, and G-CSF (1, 8, 31). In this study, we measured the responses of immunomagnetic separated CD34$^+$ umbilical cord progenitor cells with two novel cytokines, SCF and PIXY321, with and without other lineage-specific CSF.

The number of circulating CD34$^+$ progenitor cells from unfractionated umbilical cord blood was less than that from adult bone marrow ($p = 0.05$). Immunomagnetic separation using HPCA-1 incubated with rat antimouse-coated magnetic IgG1 beads resulted in an increased positive selection of CD34$^+$ cells. Positive CD34$^+$ cell separation by HPCA-1 resulted in similar lineage characteristics with respect to CD38, HLA-DR, CD33, and CD5. However, the number of CD34$^+$ cells from cord blood expressing the B-lineage antigens CD19, CD10, and CD22 was significantly reduced compared to adult bone marrow ($p = 0.01$). This reduction in B-lineage early progenitor cells may account for decreased type-specific antibody production and reduced serum Ig levels in newborns compared to adults (2).

SCF and PIXY321 induced a higher increase in cord CD34$^+$ CFU-GM colony formation compared to negative control media. PIXY321, however, did not increase cord blood or adult bone marrow CFU-GM colony formation from CD34 isolated cells compared to the combination of IL-3 + GM-CSF. This is in contrast to the comparative increase in CFU-GM formation from adult buffy coat mononuclear cell bone marrow by PIXY321 compared to GM-CSF and IL-3 (28).

The combination of growth factors that induced the highest increase in cord blood CD34$^+$ CFU-GM formation was SCF + IL-3 + IL-6. The combination of growth factors that induced the highest increase in CFU-GM formation from adult bone marrow CD34$^+$ progenitor cells, however, was SCF + IL-3 + GM-CSF. Because IL-6 acts at an earlier progenitor cell stage than GM-CSF, this data additionally suggests that cord blood contains a significantly higher population of earlier progenitor cells than does adult bone marrow.

Broxmeyer *et al.* (32) studied the *in vitro* effect of murine mast cell growth factor (c-*kit* ligand) on CFU-GM formation from CD34$^+$, HLA-DR$^+$, and CD33$^-$ adult human bone marrow sorted cells. Recombinant murine MGF induced an increase in CFU-GM colony formation from enriched human adult bone marrow progenitor cells compared to control media and was equal in potency to rhIL-3. Broxmeyer *et al.* (32) additionally demonstrated a synergistic increase in adult bone marrow CFU-GM colony formation from nonadherent lymphocyte-depleted bone marrow with rhMGF + rhGM-CSF. However, Broxmeyer was unable to demonstrate any enhancement of CFU-GM colony formation from nonadherent, lymphocyte-depleted adult bone marrow progenitor cells with a combination of MGF and G-CSF (32). These results are similar to those of our study using positive-selected CD34$^+$ cells from human umbilical cord blood.

Unfractionated and enriched murine hematopoietic cells have also been demonstrated to respond to SCF with and without IL-3, GM-CSF, and G-CSF. Migliaccio *et al.* (33) demonstrated that SCF could induce CFU-GM colony formation from serum-deprived cultures of unfractionated mouse bone marrow cells although it only represented 50% of the amount induced by G-CSF. Using more primitive murine hematopoietic progenitor cells, *i.e.* Rh123-dull sorted populations, SCF combined with IL-3 induced a more highly significant increase in CFU-GM formation on both d 10 and d 21 than either IL-3 or SCF alone (33).

Additionally, SCF has been demonstrated to stimulate blast cell colony formation from precursors from normal murine bone marrow and SCF receptors have been demonstrated to be present in high numbers on blast cells compared to immature granulocytic, monocytic, or eosinophilic precursor cells (34). Lastly, high proliferative potential colony-forming cells derived from murine bone marrow purified for early progenitors expressing the stem

cell antigen (SCA^+) and lacking terminal lineage markers (Lin^-) were highly responsive to SCF in combination with other lineage-specific hematopoietic growth factors (35). SCF in combination with either CSF-1, G-CSF, GM-CSF, or IL-3 induced an additive increase in high proliferative potential colony-forming cell formation from SCA^+Lin^- cells from enriched murine bone marrow (35).

Our present study failed to demonstrate any enhancement by PIXY321 compared to IL-3 + GM-CSF on $CD34^+$-enriched progenitor cells from umbilical cord blood or adult bone marrow. Curtis *et al.* (28) demonstrated that PIXY321 could enhance bone marrow CFU-GM formation from adherent cell and T-cell-depleted adult human bone marrow compared to the combination of IL-3 and GM-CSF. However, in the present study, using more enriched primitive populations of progenitor cells ($CD34^+$), we were unable to demonstrate any significant enhancement by PIXY321 compared to the combination of GM-CSF + IL-3. We hypothesize that the fusion protein (PIXY321) may exert a more maximal effect with more committed progenitor population than with a more primitive $CD34^+$ lineage.

These studies demonstrate that SCF has the ability to induce a significant increase in myeloid committed progenitor cell colony formation from enriched cord blood $CD34^+$ progenitor cells. SCF induces a higher increase in the CFU-GM colony formation from $CD34^+$ cord blood than do adult bone marrow progenitor cells when combined with either IL-3 or PIXY321. This enhancement by SCF, in combination with other CSF to amplify myeloid committed progenitor cell formation from cord blood-enriched progenitor cells, may be useful in the future to expand *ex vivo* cord blood progenitor cells for future bone marrow transplantation and to modulate neonatal hematopoiesis and host defense. Further studies are required to determine the *in vivo* significance of the enhancement by SCF on *in vitro* CFU-GM colony formation with lineage-specific CSF from cord blood $CD34^+$ enriched progenitor cells.

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