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Differential Responses of CD34-positive Acute Myelogenous Leukemic Blasts to the Costimulating Effects of Stem Cell Factor with GM-CSF and/or IL-3

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Stem cell factor(SCF), a c-kit ligand, has a preferential effect on the proliferation of several classes of immature hematopoietic progenitor cells in combination with GM-CSF or IL-3. To analyze the costimulatory role of SCF in leukemic growth, we investigated the effect of SCF in the presence of GM-CSF and/or IL-3 on isolated CD34-positive (CD34+) leukemic blasts from 15 patients with acute myelogenous leukemia (AML). Cultures of CD34+ cells from normal bone marrow were used as controls. When the proliferation of CD34+ AML blasts in the presence of GM-CSF and/or IL-3 were evaluated in vitro for the effects of SCF, two patterns emerged. In one pattern, CD34+ AML blasts responded with a significant increase in DNA synthesis and/or colony formation when SCF was used with GM-CSF and/or IL-3 relative to the growth with SCF alone; This result is consistent with those CD34+ bone marrow cells from normal donors. Six patients (40%) were included in this category. The addition of SCF as a single factor resulted in colony formation in all six of these cases. In the other pattern, nine of the patients (60%) had CD34+ leukemic cells whose growth with SCF plus either GM-CSF, IL-3, or GM-CSF + IL-3, was not significantly different from the growth noted in the presence of SCF alone. Among them seven cases that did not form colonies in response to SCF alone, and one case showing autocrine, background growth were included. In the six cases in which the costimulating effects of SCF were documented, CD34+ c-kit+ blasts comprised 50.5±18.7% of the CD34+ leukemic blasts - higher than 21.8±19.4% of cases in which the costimulating effect of SCF was not documented. In the cases showing high c-kit antigen expression (≥40%), SCF had a costimulatory effect in 71% (5/7) of the patients. In conclusion, our data indicate that CD34+ leukemic blasts from a good proportion of patients with AML did not respond to the costimulating effects of SCF in the presence of GM-CSF and/or IL-3, in contrast to those CD34+ bone marrow cells from normal donors. The possible use of SCF for acute leukemia must await further cytogenetic and molecular studies, which should clarify the preferential costimulating role of SCF in normal hematopoiesis.

Key Words: Acute myelogenous leukemia-CD34+ blasts-Stem cell factor

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Stem cell factor (SCF) is a ligand for the receptor encoded by the *c-kit* proto-oncogene found at the dominant White (W) locus on mouse chromosome 5 (Chabot *et al.* 1988; Geissler *et al.* 1988). SCF and the *c-kit* ligand receptor (KR) are thought to play pivotal roles in the regulation of human hematopoiesis. (Williams *et al.* 1990; Zsebo *et al.* 1990a). SCF has a preferential effect on the proliferation of several classes of immature

hematopoietic progenitor cells including the colony-forming unit-Blast (CFU-Blast), the high proliferative potential colony-forming cells (HPP-CFC) and the long-term bone marrow culture-initiating cell (LTBMC-IC) in combination with specific late-lineage cytokines such as interleukin-3 (IL-3) or granulocyte-macrophage colony-stimulating factor (GM-CSF) (Zsebo *et al.* 1990b; Bernstein *et al.* 1991; Broxmeyer *et al.* 1991; McNiece *et al.* 1991).

Although leukemic progenitor cells in vivo may have apparently escaped normal regulation by stimulatory and inhibitory factors, clonogenic leukemic cell growth of primary leukemias in vitro has been shown to be dependent on hematopoietic growth factors in most cases (Griffin and Löwenberg, 1986; Delwel *et al.* 1988). There are only a limited number of current studies evaluating the effects of SCF on clonogenic leukemic myeloblasts in vitro (Ikeda *et al.* 1991; Wang *et al.* 1991; Carlesso *et al.* 1992). The addition of SCF as a single factor resulted in only minimal colony growth and DNA synthesis by unseparated clonogenic leukemic precursor cells (Ikeda *et al.* 1991; Goselink *et al.* 1992; Tafuri *et al.* 1993); the SCF-induced proliferation of acute myelogenous leukemia (AML) cells was significantly augmented by the addition of GM-CSF or IL-3. However, growth of leukemic cells in vitro to SCF in the presence or absence of GM-CSF and/or IL-3 varied from patient to patient (Goselink *et al.* 1992; Pietsch *et al.* 1992; Ikeda *et al.* 1993). In a proportion of AML patients, cells did not respond at all to SCF with GM-CSF and/or IL-3 (Pietsch *et al.* 1992; Maze *et al.* 1993). So far, only a few studies have been reported on the growth characteristics of AML CD34-positive ($CD34^+$) cells. Leukemic clonogenic precursors in short-term and long-term cultures were shown to be $CD34^+$ (Silvestri *et al.* 1992; Ikeda *et al.* 1993), and the proliferative potential for AML cells to SCF in the long-term culture was found to be highly related to the coexpression of CD34 and *c-kit* (Ikeda *et al.* 1993). To analyze the potential role of SCF in the growth of leukemic progenitor cells in vitro in the presence of late-acting hematopoietic growth factors, we investigated the costimulating effects of SCF with GM-CSF and/or IL-3 on the proliferation of isolated CD34+ leukemic blasts from patients with AML and compared with those of normal BM CD34+ cells. By using this method, the possibility of altering the growth kinetics of AML progenitor cells by endogenous colony stimulating factors from accessory cells can be avoided. If the costimulating effect of SCF is noted preferentially on normal CD34+ bone marrow cells, SCF may be useful in the settings of stem cell transplantation for patients with acute leukemia.

MATERIALS AND METHODS

Patients and normal donors

Fifteen patients with AML receiving no drug treatment prior to the study, were included in this investigation (Table 1). They were classified as 3 patients with M1, 8 patients with M2, 2 patients with M4, 1 patient with M5, and 1 patient with M6 in accordance to the French-American-British (FAB) classification (Bennet *et al.* 1985). Their marrow samples were obtained with informed consent. Normal marrow samples for allogeneic bone marrow transplantation were also obtained with informed consent from 10 healthy marrow donors.

Preparation of CD34-positive (CD34+) cell suspension

The mononuclear cell (MNC) fraction from aspirated bone marrow was collected using the Lymphoprep (Nyegaard, Oslo, Norway) (density 1.077 g/cm^3) density gradient centrifugation at 400*g* for 30 minutes. From which the T-cells were removed using CD2-conjugated immunomagnetic beads (Dynabeads M-450; Dynal A.S., Oslo, Norway). Cytomorphological staining determined that more than 90% of the cells were leukemic in the BM MNC suspension from patients with AML. Monocyte in normal bone marrow samples was further removed by plastic adherence (Delwel *et al.* 1988), which was applied for 1 hour at 37℃ before isolating the CD34+ cells. The CD34+

cells were isolated through positive selection using a slightly modified version of Miltenyi's *et al* (1990) use of immunomagnetic beads. A brief summary of the procedure would be as follows. After adjusting the cell concentration at 4×10^8 nucleated cells/ml, resuspend the cells in a 300 μL phosphate buffer saline solution containing 0.5% bovine serum albumin (PBS/0.5% BSA) per 10^8 cells. Next, add 100 μL of F_C-receptor blocking agent (human IgG) and 100 μL of CD34 antibody (clone 8G12, IgG1) per 10^8 cells, and mix gently. After a 15 minute incubation period at 4℃, carefully wash the cells once in a 400 μL buffer. Add 100 μL of colloidal supermagnetic MACS microbead (Miltenyi Biotec GmbH, Germany) per 10^8 cells. Mix gently and incubate for 15 minutes at 4℃. After washing the cells, fill the MiniMACS column (Miltenyi Biotec GmbH, Germany) by pipetting 500 μL of buffer on top, and discard the effluent material. Apply the cells to a prefilled MiniMACS column (type MS, without flow resistor), and allow the cells to pass through the column and then wash it 4 times with the 500 μL buffer. Remove the column from the separator, and place the column on a suitable tube. Pipette 1 mL of buffer on top of the column and elute any retained cells using a plunger (CD34+ fraction). For increased sensitivity, this fraction can be passed through the column a second time. The CD34+ cells are then collected in a tube containing 1 mL of IMDM (Gibco, Grand Island, NY, USA) comprised of 10% fetal bovine serum(FBS; Gibco, Grand Island, NY, USA). The purity and the phenotypic analysis of the isolated CD34+ cells were then assessed by flow cytometry.

Immunophenotyping of CD34-positive cells

A two-color immunophenotyping procedure using directly conjugated MoAbs was performed on the study cells. The portion of CD34+ cells coexpressing CD33, HLA-DR, CD38 and *c-kit* was determined by simultaneously incubating cells with anti-HPCA-2 (CD34)-FITC in combination with either anti-Leu-M9(CD33)-PE, anti-Leu-17(CD38)-PE, anti-HLA-DR-PE (all from Becton Dickinson), or anti-*c-kit*-PE (from Im-munotech). Cells stained with the appropriate directly conjugated isotype antibodies were used as controls. Over 10,000 entities were collected in listmode on a FACStar plus (Becton Dickinson, Mountain View, CA) equipped with an argon-ion laser, and analyzed using LYSIS II software (Becton Dickinson). The proportion of CD34+ cells coexpressing the individual antigens was determined by assessing only cells within the CD34+ gated population.

Recombinant human hematopoietic growth factors

Recombinant SCF, and recombinant human IL-3 came from Genzyme (Cambridge, MA, USA) and were used at a concentration of 50 ng/ml, and 50 ng/ml, respectively. Recombinant human GM-CSF, kindly provided by Lucky BioTech (Seoul, Korea), was added to each culture at a concentration of 5 ng/ml. Optimal concentration for SCF, IL-3, and GM-CSF was chosen after a preliminary dose-response study of thymidine uptake and colony formation from the normal marow cells (GM-CSF and IL-3), and from the M07E cell line (SCF).

^{3}H-Thymidine (^{3}H-TdR) incorporation

The following ^{3}H-TdR incorporation assay (Delwel *et al.* 1988) was used to determine the mitogenic activity of SCF. Isolated CD34+ cells were plated in a concentration of 5×10^3 cells/ml on 96-well flat bottom microtiter plates containing an optimal concentration of hematopoietic growth factors (HGFs). The combination of HGFs used are as follows: GM-CSF, IL-3, SCF, GM-CSF+IL-3, GM-CSF+SCF, SCF+IL-3, and SCF+GM-CSF+IL-3. After incubation for 7 days at 37℃ in a 5% CO2/95% atmosphere, the cells were exposed to a 4-hour pulse of 0.5uCi 3H-thymidine (25 Ci/mmol; Amersham, Braunschweig, Germany). Finally, the radioactivity was measured on a scintillation counter (Packrad, Frankfurt, Germany). All assays were performed in triplicate.

Colony assay

Isolated CD34+ cells were grown at a con-

centration of 2×10^3/ml in methylcellulose composed of IMDM, which in turn consisted of 1.0% methylcellulose (Methocel 4000 cps, Fluka, Germany) supplemented with 20% heat-inactivated FBS, 0.8% dialyzed bovine serum albumin(BSA) (Sigma, St. Louis, MO), 10^{-4}M 2-mercaptoethanol (Sigma) and HGFs. Various combinations of HGFs were used in thymidine incorporation assay. Cells were plated in 35× 10 mm tissue culture dishes (Costar), and then incubated in a fully humidified incubator at 37℃, 5% CO_2. Colonies were enumerated after 14 days of incubation.

Statistics

Results are expressed as the mean±SD. Levels of significance between samples are determined using the non-parametric (Mann-Whitney) test.

RESULTS

Purification and analysis of CD34+ cells

According to flow cytometry analysis, the AML CD34+ cells were purified from 35.9± 29.0% to 94.6±2.8% with a 45.5±30.4% yield (Table 1). Normal BM CD34+ cells had the purity of 86.2±14.3%. Double-color fluorescence analysis demonstrated that the majority of purified CD34+ AML blasts coexpressed HLA-DR(87.2±20.6%) and CD38 (86.2±15.5%). The majority of normal marrow CD34+ cells also coexpressed HLA-DR, and CD38. CD33 antigen was detectable in 49.5±35.9% of CD34+ AML blasts. We found that *c-kit* expression of CD34+ AML blasts averaged 33.3±28.6% with a range from 0.2% to 89.9%. In normal bone marrow cell samples, 24.4±8.3% of CD34+ cells coexpressed *c-kit*.

Table 1. Phenotype analysis of CD34-positive acute myelogenous leukemic cells after immunomagnetic isolation

UPN	Patient Name	FAB	CD34+ (Pre)*	CD34+/ CD33+(%)	CD34+/ HLA-DR+(%)	CD34+/ CD38+/(%)	CD34+/ *c-kit*+(%)
1.	JYM	M1	34.1	19.6	97.8	96.8	53.6
2.	JYJ	M2	45.1	ND	ND	ND	67.5
3.	KKS	M1	86.4	1.2	45.6	82.5	0.2
4.	YKH	M4	9.8	78.5	99.5	50.0	42.5
5.	CYW	M2	61.8	51.8	98.6	99.9	20.7
6.	PMR	M2	79.4	2.9	96.8	72.0	3.7
7.	OJR	M5	15.6	73.5	89.5	95.1	73.1
8.	KYO	M4	1.7	14.9	95.5	91.3	21.0
9.	KHS	M2	5.2	77.5	93.3	96.1	89.9
10.	LNS	M2	81.2	95.2	99.7	99.9	8.9
11.	KKJ	M1	38.0	93.1	46.1	92.3	44.1
12.	KJK	M6	11.2	36.9	97.3	72.8	1.0
13.	SSO	M2	22.4	ND	ND	ND	45.4
14.	KYS	M2	12.5	ND	ND	ND	21.0
15.	KSM	M2	34.5	ND	ND	ND	7.5
Mean			35.9	49.5	87.2	86.2	33.3
SD			29.0	35.9	20.6	15.5	28.6

UPN: Unique patient number
*CD34+ (Pre): percentage of CD34-positive acute myelogenous leukemic blasts in bone marrow mononuclear cells before purification of CD34-positive cells
% of cells expressing the indicated surface molecule
ND: not done

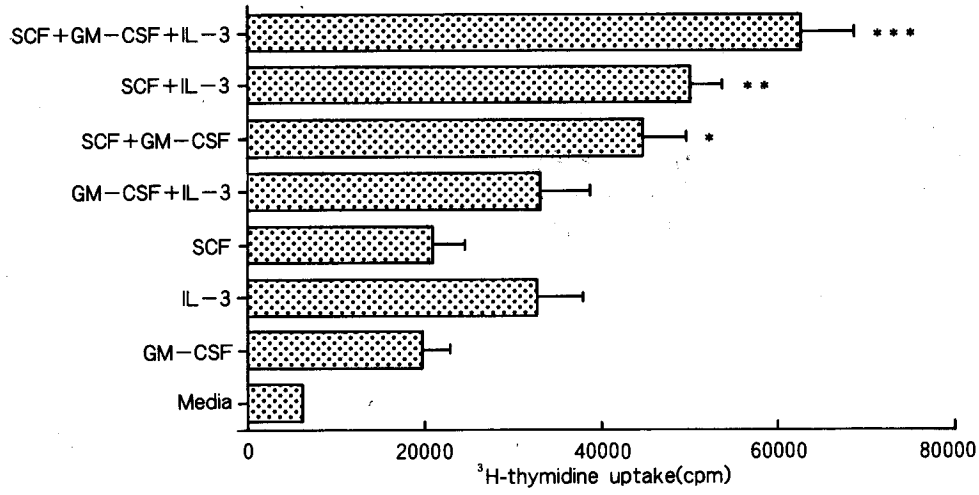

Fig. 1. *Effect of SCF alone and in the presence of GM-CSF and/or IL-3 on thymidine uptake from isolated bone marrow CD34+ cells from normal donors. Results reflect the mean number of ^{3}H-thymidine incorporation(cpm)+SD of ten experiments after 7-day incubation. *, SCF vs SCF+GM-CSF, $p<0.001$; **, SCF vs SCF+IL-3, $p<0.001$; ***, SCF vs SCF+GM-CSF+IL-3, $p<0.001$.*

Effects of SCF on the proliferation of CD34+ bone marrow cells from normal donors in the presence or absence of GM-SCF and/or IL-3

The influence of SCF upon DNA synthesis and colony formation of CD34+ normal bone marrow cells was studied in the absence or presence of GM-CSF and/or IL-3 (Fig. 1). SCF alone stimulated DNA synthesis (stimulation index above 1.8) in eight out of 10 cases (data not shown). Moreover, SCF alone gave rise to colony-forming unit granulocyte-macrophage (CFU-GM) colonies in eight out of 10 cases. If the number of colonies grown in the presence of GM-CSF was considered as 100% growth, the addition of SCF as a single factor resulted in 75.1±28.2% of colony growth observed with GM-CSF (Fig. 2). When SCF was combined with GM-CSF and/or IL-3, a marked enhancement of DNA synthesis and colony formation was observed in all of the cases studied (Fig. 1, Fig. 2). SCF with GM-CSF ($p<0.001$), IL-3 ($p<0.001$), and GM-CSF plus IL-3 ($p<0.001$) had an additive effect upon DNA synthesis and colony formation as opposed to the culture initiated with SCF alone. Moreover, the combination of SCF + GM-CSF + IL-3 induced a significant increase in DNA synthesis as opposed to the combination of GM-CSF + IL-3 ($p<0.001$), SCF + GM-CSF ($p<0.005$), and SCF + IL-3 ($p<0.05$).

Effects of SCF on the proliferation of CD34+ blasts from patients with AML in the presence or absence of GM-SCF and/or IL-3

The influence of SCF on DNA synthesis and colony formation in CD34+ AML cells was studied in the absence or presence of GM-CSF and/or IL-3 (Table 2). SCF stimulated DNA synthesis in isolated CD34+ leukemic blasts in ten out of 15 cases (66.7%). In one case that already exhibited high spontaneous proliferation (UPN #6), SCF did not augment proliferation. SCF stimulated colony formation in eight out of 15 cases (53.3%), while GM-CSF stimulated colony formation in nine cases. SCF did not stimulate CD34+ AML blasts to form colonies in patients who demonstrated no colony formation under the stimulation of GM-CSF. The addition of SCF as a single factor resulted in 76.8±36.8% of colony growth observed with GM-CSF. Aside from

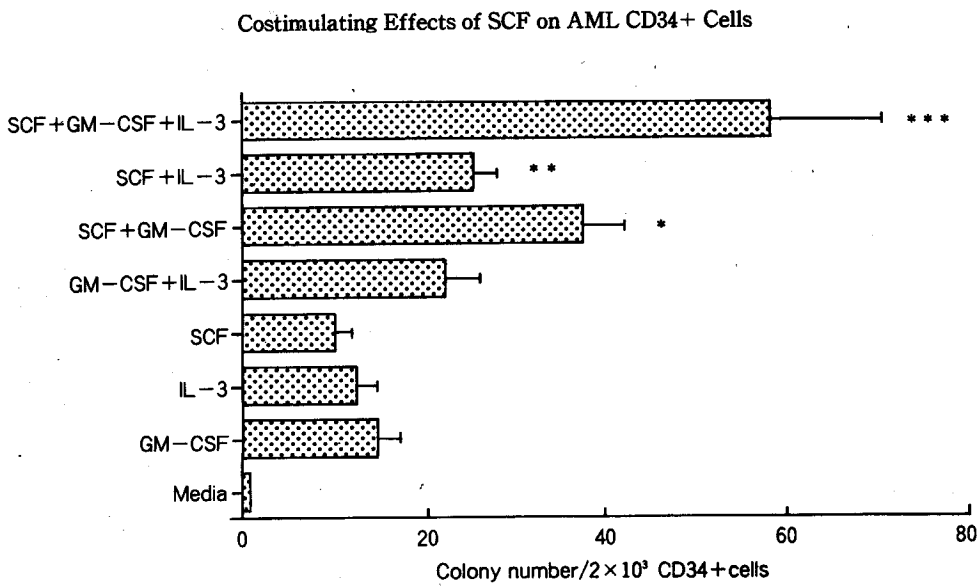

Fig. 2. *The effect of SCF alone and in the presence of GM-CSF and/or IL-3 on colony formation from isolated bone marrow CD34+ cells from normal donors. CD34+ cells were plated at 2,000 cells per ml in methylcellulose culture media. Results reflect the mean number of colonies+SEM of 10 experiments scored after 14 day incubation. *, SCF vs SCF+GM-CSF, $p<0.001$; **, SCF vs SCF+IL-3, $p<0.001$; ***, SCF vs SCF+GM-CSF+IL-3, $p<0.001$.*

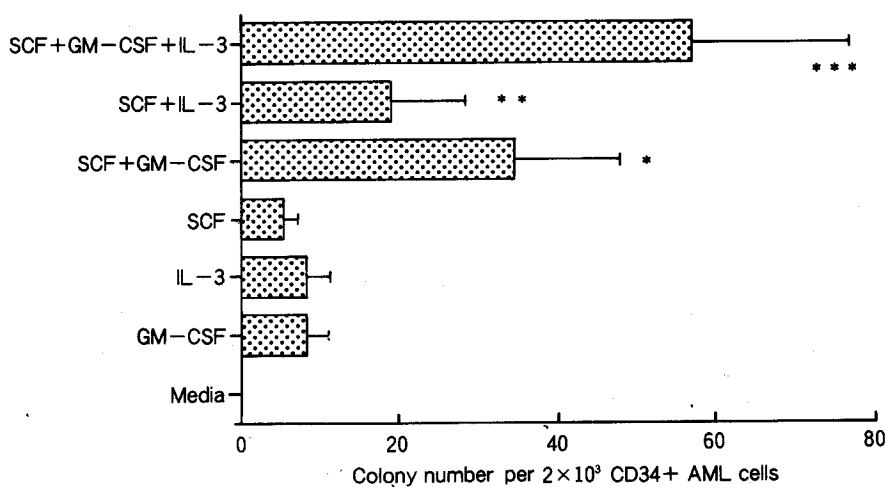

Fig. 3. *The effect of SCF alone and in the presence of GM-CSF and/or IL-3 on colony formation from isolated CD34+ AML blasts from six SCF costimulating responders. Results reflect the mean number of colonies+SD scored after 14 days of incubation. *, SCF vs SCF+GM-CSF, $p<0.05$; **, SCF vs SCF+IL-3, $p<0.05$; ***, SCF vs SCF+GM-CSF+IL-3, $p<0.05$.*

one (UPN #12) patient, SCF was less potent than GM-CSF for colony formation. In the absence of added growth factors, cells from only one patient (UPN #12) formed background colonies.

When the proliferation of CD34+ AML blasts in the presence of GM-CSF and/or IL-3 were evaluated in vitro for the effects of

Table 2. Response of CD34+ acute myelogenous leukemic blasts to the costimulating effects of stem cell factor

Unique Patient Number		Growth of CD34+ AML cells stimulated with						
		Nothing	GM-CSF	IL-3	SCF	SCF+ GM-CSF	SCF+ IL-3	SCF+ GM-CSF+IL-3
Costimulating Responders								
1	3H-TdR uptake	687	3586	912	706	7907	1319	10725
	No. of colony	0	5	2	3	10	3	8
2	3H-TdR uptake	620	2652	3093	1290	15504	33716	36996
	No. of colony	0	5	3	3	13	5	18
4	3H-TdR uptake	6181	45028	25115	14033	61950	44076	73853
	No. of colony	0	3	0	2	20	0	23
7	3H-TdR uptake	587	1038	13824	2943	719	17953	736
	No. of colony	0	5	17	3	15	60	112
13	3H-TdR uptake	497	1149	1388	383	1526	4050	6956
	No. of colony	0	22	8	13	75	23	90
14	3H-TdR uptake	7286	4939	6854	4568	4489	1497	5989
	No. of colony	0	8	18	7	75	23	90
Costimulating Nonresponders								
3	3H-TdR uptake	524	6737	800	4177	3308	2660	2502
	No. of colony	0	0	0	0	0	0	0
5	3H-TdR uptake	171	627	3532	2111	469	3636	476
	No. of colony	0	3	0	2	3	0	4
6	3H-TdR uptake	10021	5501	16843	12230	1850	21864	1683
	No. of colony	625	0	0	0	0	0	0
8	3H-TdR uptake	0	979	1111	4171	947	515	1176
	No. of colony	0	3	0	0	5	0	4
9	3H-TdR uptake	707	1001	966	2391	588	2535	578
	No. of colony	0	0	0	0	0	0	1
10	3H-TdR uptake	217	7291	170	4966	2865	2472	1118
	No. of colony	0	0	0	0	0	1	1
11	3H-TdR uptake	1830	3193	10211	7347	6421	11176	9159
	No. of colony	0	0	0	0	0	0	0
12	3H-TdR uptake	2853	1175	10285	8214	1566	2222	2028
	No. of colony	125	190	175	262	278	273	250
15	3H-TdR uptake	1111	755	1623	1610	1068	1736	1161
	No. of colony	0	0	0	0	0	0	0

AML: Acute myelogenous leukemia

*[1]The terms costimulating responders and nonresponders refer to patients whose DNA synthesis and/or colony formation responded or did not respond to the costimulating effect of stem cell factor(SCF) respectively.

*[2]CD34+ AML Blasts were cultured in IMDM culture medium (5×10^3 cells/mL) in the presence or absence of various CSFs (see Materials and Methods). Cells were pulsed with [^{3}H]thymidine for the last 4 hours of 7-day incubation.

*[3]The CD34+ AML blasts were incubated at a concentration of 2×10^3 cells/mL with individual CSFs. After 14 days of incubation, colonies ($\geq$40 cells/group) were counted.

*[4]Data are shown as the median value in triplicate experiments.

SCF, two patterns emerged. In one pattern, bone marrow CD34+ leukemic cells responded with a significant increase in DNA synthesis and/or colony formation when SCF was used with GM-CSF and/or IL-3 when compared to growth with SCF alone; This result is consistent with those CD34+ bone marrow cells of normal donors. Six patients(40%) were included in this category (Table 2). SCF combined with GM-CSF and/or IL-3 significantly increased the number of colonies as opposed to the effects of SCF alone ($p<0.05$), of GM-CSF alone ($p<0.05$), or of IL-3 alone ($p<0.05$) (Fig. 3). Of the 6 cases, SCF synergized with GM-CSF in four cases, with IL-3 in four cases, and with GM-CSF plus IL-3 in four cases for DNA synthesis. In these 4 cases, SCF in combination of GM-CSF and IL-3 appeared to be highly potent mitogenic stimulus of CD34+ cells. In one case, SCF plus IL-3 resulted in the highest levels of DNA synthesis when compared to the other SCF-cytokine combinations. Except for one case (UPN #14), the costimulatory effects of SCF, when combined with GM-CSF and/or IL-3, was seen in the colony assay in essentially the same cases that synergy in the thymidine uptake was seen. All of these cases were SCF-responders for colony formation.

In the other pattern, 9 of the patients (60%) had CD34+ leukemic cells whose growth with SCF plus either GM-CSF, IL-3, or GM-CSF + IL-3 was not significantly different from the growth noted in the presence of SCF alone, GM-CSF alone, or IL-3 alone. Among them seven cases that did not form colonies in response to SCF alone, and one case (UPN # 12) showing autocrine, background growth were included.

We evaluated the responsiveness of cells to the costimulating effect of SCF from selected patients by analyzing the coexpression of CD33, HLA-DR, CD38, and *c-kit* antigen on the same CD34+ leukemic blasts used in this study (Table 1). Expression of CD33, HLA-DR, and CD38 did not correlate to the costimulating effects of SCF with GM-CSF and/or IL-3. In five cases in which the costimulating effects of SCF was documented, CD34+ *c-kit* + blasts comprised 50.5±18.7% (42.5~73.0%) of the CD34+ leukemic blasts; these tend to be higher than −21.8±19.4% (1.0 ~89.9%) −the cases in which the costimulating effect of SCF was not documented (Table 1, Table 2). In the cases showing high *c-kit* antigen expression (≥40%), SCF had a costimulatory effect in 71% (5/7) of the patients.

DISCUSSION

In the presence of GM-CSF and/or IL-3, the potent costimulating effects of SCF on early hematopoietic progenitor cells in vitro, and the blood cell-stimulating effects of SCF in vivo in animal models (Molinneux *et al.* 1991; Ulich *et al.* 1991; Andrews *et al.* 1992) suggest the usefulness of SCF in certain clinical disorders including acute leukemia requiring enhanced hematopoiesis. There are only a limited number of studies evaluating the effects of SCF in vitro on hematopoietic progenitor cells from patients with leukemia (Ikeda *et al.* 1991; Wang *et al.* 1991; Goselink *et al.* 1992; Pietsch *et al.* 1992). *c-kit* mRNA and encoded surface expression have been described from a number of patients with acute leukemia. In most, if not all cases, it has been reported that in a semisolid medium clonogenic cells from these patients proliferate in response to the effects of SCF when combined with a colony-stimulating factor such as GM-CSF (Carlesso *et al.* 1992; Goselink *et al.* 1992). In one report, some variability in res-ponsiveness to SCF has been seen in clonogenic cells from patients with AML, which res-ponded-but to a lesser degree than did cells from patients with chronic myelogenous leukemia and from normal marrow donors(Goselink *et al.* 1992). In another report (Pietsch *et al.* 1992), cells from a few AML patients did not respond. All the studies described above were done for the unseparated, heterogenous leukemic cell population. Therefore, evaluation of growth pattern in vitro of rather homogenous, purified leukemic blasts, which have characteristics of progenitor cells, to costimulating effects of SCF should be ensued. It was shown that in short-term and long-term cultures, leukemic clono-genic pre-

cursors were CD34-positive (Silvestri *et al.* 1992; Ikeda *et al.* 1993); furthermore, the proliferative potential of AML cells to SCF in the long-term culture was found to be highly related to the coexpression of CD34 and *c-kit* (Ikeda *et al.* 1993). To analyze the potential role of SCF on the growth of leukemic progenitor cells in vitro, we investigated the costimulating effects of SCF with GM-CSF and/or IL-3 upon the proliferation of isolated CD34+ leukemic blasts from patients with AML, and compared those with normal BM CD34+ cells.

As previously stated (Bernstein *et al.* 1991; Broxmeyer *et al.* 1991; McNiece, 1991), SCF synergizes with GM-CSF and/or IL-3 when acting on normal BM CD34+ hematopoietic progenitor cells. When SCF is combined with GM-CSF and/or IL-3, a marked enhancement of DNA synthesis and colony formation was observed in all the cases studied.

When the proliferation of CD34+ AML blasts in the presence of GM-CSF and/or IL-3 were evaluated in vitro for the effects of SCF, two patterns emerged. In one pattern, bone marrow CD34+ leukemic cells responded with a significant increase in DNA synthesis and/or colony formation when SCF was used with GM-CSF and/or IL-3 relative to the growth with SCF alone; This result is consistent with those CD34+ bone marrow cells of normal donors. Six patients(40%) were included in this category. Costimulatory effects of SCF in combination GM-CSF and/or IL-3 were also seen in colony assay in essentially the same cases that also showed synergy in the thymidine uptake assay except one case. The addition of SCF as a single factor resulted in colony formation in all six of these cases. In the other pattern, nine of the patients(60%) had CD34+ leukemic cells whose growth with SCF plus either GM-CSF, IL-3, or GM-CSF + IL-3 was not significantly different from that growth noted in the presence of SCF alone, GM-CSF alone, or IL-3 alone. Our study revealed that the costimulating effect of SCF on CD34+ AML blasts in the presence of GM-CSF and/or IL-3 occured where colonies were formed with SCF, whereas cells lacking the responsiveness to SCF for colony formation might react defectively with GM-CSF or IL-3.

It is not clear why CD34+ leukemic cells from a good proportion(60%) of patients with AML did not respond to the costimulating effects of SCF. Lack of response may be explained in a number of ways. First, the CD34 + blasts that does not respond may lack SCF-receptors, or may have a low level of *c-kit* expression. Although it was shown that the proliferative response of the unseparated blasts did not correlate with the amount of bound 125I-SCF (Pietsch *et al.* 1992), the proliferative response of CD34+ AML cells tend to correlate to the extent of *c-kit* expression on the CD34+ leukemic blasts in our study. In five cases in whom the costimulating effects of SCF was documented, CD34+ *c-kit*+ blasts comprised 50.5±18.7% of the CD34+ leukemic blasts, which tend to be higher than the 21.8± 19.4% in cases where the costimulating effect of SCF was not documented. All of the responders had CD34+ leukemic blasts showing high c-kit antigen expression (≥40%), while only 20% of the nonresponder had CD34+ cells showing high *c-kit* expression. Because the vast majority of CD34+ c-kit+ leukemic blasts evaluated in our study were HLA-DR+, it may be suggested that the costimulating effects of SCF were more prominent in CD34+ HLA-DR+ *c-kit*+ leukemic myelogenous blasts than in CD34+ HLA-DR+ c-kit- blasts, the more differentiated phenotypic subset. In the future, it will become significant to evaluate costimulating ef fects of SCF on more primitive subsets, such as CD34+ HLA-DR-, *c-kit*+ leukemic blasts (Brandt *et al.* 1992; Briddel *et al.* 1992; Steen *et al.* 1994), which would investigate the significance of the SCF-*c-kit* axis in acute myelogenous leukemia. Second, in contrast to normal CD34+ cells, there may be an abnormal ligand-receptor interaction, where there is a dysregulation of the c-kit receptor post-transcriptional regulation and the signal transduction in leukemic CD34+ blasts. Third, the differential costimulating effect of SCF may be mediated by interactions of other cytokines such as IL-4, or TNF(Budel *et al.* 1993). Fourth, costimulation by dual factors probably triggers additional subsets of CD34+ leukemic cells that does not respond

to the individual factors. The synergistic proliferative effects of SCF with other cytokines such as GM-CSF or IL-3 appear to be at least partially explained by actions on immediately early gene expression (*c-fos, jun-B, c-egr, and c-myc)* (Horie and Broxmeyer, 1993). Synergistic proliferation of cells in responses to SCF plus GM-CSF or IL-3 does not appear to involve SCF-induced upregulation of GM-CSF or IL-3 receptor number or altered sensitivity to hematopoietic growth factor stimulation (Hendrie *et al.* 1991; Budel *et al.* 1993).

In conclusion, CD34+ leukemic blasts from a good proportion of patients did not respond to the costimulating effects of SCF with GM-CSF and/or IL-3. However, results do suggest that SCF, in combination with late-acting hemato-poietic growth factors such as GM-CSF and/or IL-3, may be useful in the future to preferentially expand ex vivo and in vivo normal hematopoietic stem cell in the settings of stem cell transplantation for patients with acute leukemia. The discovery that SCF has preferential costimulatory effects on CD34+ AML cells with high levels of c-kit expression and colony-forming capacity in response to SCF should be further investigated. The possible use of SCF in acute leukemia must await further cytogenetic and molecular studies, which should clarify the preferential role of SCF in normal hematopoiesis.

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