

# Cytotoxic Lymphocyte Maturation Factor (Interleukin 12) Is a Synergistic Growth Factor for Hematopoietic Stem Cells

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## Summary

The recently cloned cytotoxic lymphocyte maturation factor (interleukin 12 [IL-12]) has been described as a growth factor for mature lymphocytes. The present study investigated whether purified recombinant murine IL-12 (rMuIL-12) also could affect the proliferation of primitive bone marrow progenitor cells. Using a population of Lin<sup>-</sup>Sca-1<sup>+</sup> murine bone marrow stem cells, we now demonstrate that IL-12 is a potent synergistic factor for primitive hematopoietic stem cells. The synergy of IL-12 was observed in single-cell cloning assays, demonstrating that its effects are directly mediated. Specifically, IL-12 enhanced stem cell factor-induced myelopoiesis of Lin<sup>-</sup>Sca-1<sup>+</sup> cells sevenfold, and synergized with colony-stimulating factors (CSFs) to induce proliferation of Lin<sup>-</sup>Sca-1<sup>+</sup> stem cells. IL-12 increased the number of responding progenitor cells as well as the size of the colonies formed. IL-12 also increased colony formation of high proliferative potential colony-forming cells with multiple CSF combinations. The effects of IL-12 were concentration dependent with a 50% effective dose of 2–20 and 20–200 ng/ml, resulting in maximum stimulation. Furthermore, a neutralizing anti-IL-12 antibody blocked the synergistic effects of rMuIL-12. In addition, IL-12 was found to have synergistic effects on more committed bone marrow progenitors as well. Our results therefore suggest that in addition to being a potent lymphopoietic stimulator, IL-12 is a regulator of the growth of hematopoietic stem cells and their myeloid progeny.

Mature hematopoietic cells are mostly short-lived and continuously renewed by pluripotent hematopoietic stem cells capable of producing cells of all hematopoietic lineages (1–3). While committed progenitor cells can proliferate in response to individual hematopoietic growth factors (HGFs)<sup>1</sup> in vitro, the most immature hematopoietic stem cells are quiescent and can only be induced to optimal proliferation as a result of synergy between multiple HGFs (4–9). A physiological explanation for this restrictive responsiveness could be that mobilization of stem cells should occur only under extraordinary conditions, and thereby protect the stem cell pool from exhaustion. Although our knowledge about HGFs regulating stem cell proliferation is limited, a newly identified stem cell factor (SCF) has been demonstrated to be the most potent stem cell growth factor in cooperation with the CSFs, IL-1 and IL-6 (4–6, 10, 11).

The Lin<sup>-</sup>Sca-1<sup>+</sup> cells in murine bone marrow contain the

most immature hematopoietic stem cells characterized to date (2, 12, 13). As few as 100 of these cells can rescue 50% of lethally irradiated mice and long-term reconstitute all lineages in the blood (2, 12, 13). Although some HGFs have been demonstrated to stimulate in vitro proliferation of Lin<sup>-</sup>Sca-1<sup>+</sup> stem cells (4–6), defining novel stem cell growth factors still represents a major challenge. To investigate whether cytotoxic lymphocyte maturation factor (CLMF; also called NK cell stimulatory factor or IL-12), a cytokine previously demonstrated to stimulate only mature lymphoid cells (14–17), also might affect the proliferation of murine hematopoietic stem cells, Lin<sup>-</sup>Sca-1<sup>+</sup> bone marrow cells were sorted and investigated for IL-12 responsiveness both in the presence and absence of other HGFs.

## Materials and Methods

**Growth Factors and Antibodies.** Purified recombinant human (rHu)G-CSF, recombinant murine (rMu)GM-CSF, and recombinant rat (rr)SCF were generously supplied by Dr. Ian K. McNiece (Amgen Corp., Thousand Oaks, CA). rMuIL-3 was purchased from Promega Corp. (Madison, WI). Purified rHuCSF-1 was kindly provided by Dr. Michael Geier (Cetus Corp., Emeryville, CA).

<sup>1</sup> Abbreviations used in this paper: CLMF, cytotoxic lymphocyte maturation factor; HGF, hematopoietic growth factor; HPP-CFC, high proliferative potential colony-forming cell; rHu, recombinant human; rMu, recombinant murine; rr, recombinant rat; SCF, stem cell factor.

Purified rMuIL-12 was a generous gift from Dr. Stan Wolf (Genetics Institute, Cambridge, MA). Purified rHuIL-1 $\alpha$  was kindly supplied by Hoffmann-La Roche, Inc. (Basel, Switzerland), and rHuIL-7 by Steven Gillis (Immunex Corp., Seattle, WA). Purified rMuIL-4 and rHuIL-2 were purchased from Genzyme (Cambridge, MA). Unless otherwise indicated, all growth factors were used at predetermined optimal concentrations: rMuGM-CSF, 20 ng/ml; rHuG-CSF, 20 ng/ml; rMu IL-3, 20 ng/ml; rHuCSF-1, 50 ng/ml; rSCF, 100 ng/ml; rHuIL-1 $\alpha$ , 20 ng/ml; rHuIL-7, 100 ng/ml; rMuIL-4, 20 ng/ml; rHuIL-2, 200 U/ml. A monoclonal rat anti-mouse IL-12 was generously provided by Dr. Richard Chizzonite (Hoffmann-La Roche, Inc., Nutley, NJ) and an isotype-matched antibody was used as a control.

**Enrichment and Purification of Murine Bone Marrow Progenitor Cells.** Lin<sup>-</sup> bone marrow cells were isolated from normal C57BL mice, according to a previously described protocol (20). Briefly, low density bone marrow cells were obtained using lymphocyte separation medium (Nycomed, Oslo, Norway). Cells were washed twice in IMDM (Gibco, Paisley, UK) and resuspended in IMDM supplemented with 20% FCS (Sera Lab, Sussex, UK). The cells were incubated at 4°C for 30 min in a cocktail of predetermined optimal concentrations of antibodies: RA3-6B2 (B220 antigen; Pharmingen, San Diego, CA), RB6-8C5 (GR-1 antigen; Pharmingen), MAC-1 (Serotec; Oxfordshire, UK), Lyt-2 (CD8; Becton Dickinson & Co., Sunnyvale, CA), and L3T4 (CD4; Pharmingen). Cells were washed twice and resuspended in complete IMDM. Sheep anti-rat IgG (Fc)-conjugated immunomagnetic beads (Dyna, Oslo, Norway) were added at a cell/bead ratio of 1:20, and incubated at 4°C for 30 min. Labeled (Lin<sup>+</sup>) cells were removed by a magnetic particle concentrator (Dyna), and the Lin<sup>-</sup> cells recovered from the supernatant.

Lin<sup>-</sup>Sca-1<sup>+</sup> cells were purified as described by others (2, 12). Briefly, 4–6  $\times 10^7$  Lin<sup>-</sup> cells were resuspended per milliliter of complete IMDM and incubated for 30 min on ice with either FITC-conjugated rat anti-mouse Ly-6A/E antibody (Pharmingen) or an isotype-matched control antibody. The cells were washed twice, and Lin<sup>-</sup>Sca-1<sup>+</sup> cells were sorted on an Epics Elite Cell Sorter (Coulter Electronics, Hialeah, FL) equipped with a 488-nm tuned argon laser set to give a power of 15 mW, with a rate of 1,500–2,000 cells/s. Lin<sup>-</sup> cells falling into median right angle scatter and median to high forward scatter were analyzed for Sca-1 expression, and cells falling into both regions were selected. Light scatter was collected through a 488-nm band pass filter and FITC fluorescence was collected through a 488-nm-long pass filter and 525-nm band pass filter. The final recovery of Lin<sup>-</sup>Sca-1<sup>+</sup> cells was 0.05–0.1% of the unfractionated bone marrow.

**Soft Agar Colony Formation.** A modification of the method described by Stanley et al. (18) was used to measure colony formation of murine bone marrow progenitors in vitro. Low density and Lin<sup>-</sup> bone marrow cells were plated at 5  $\times 10^4$  and 2  $\times 10^4$  cells/ml, respectively, in IMDM containing 20% FCS and 0.3% sea plaque agarose (FMC Bioproducts, Naperville, IL) and incubated at 37°C in 5% CO<sub>2</sub> for 7 d and scored for colony growth (>50 cells).

**High Proliferative Potential Colony-forming Cell (HPP-CFC) Assay.** The HPP-CFC assay was performed as previously described by others (19).  $\alpha$  modification of Eagle's MEM (MEM-Alpha; Gibco Laboratories, Grand Island, NY) supplemented with 20% FCS (Sera Lab) was used for all HPP-CFC assays. Cytokines were incorporated into a 1-ml underlayer at a maximum of 3% of the culture volume, and cells were incorporated into a 0.5-ml overlay. The final concentration of sea plaque agarose was 0.5% in the underlayer and 0.3% in the overlay. HPP-CFC colonies were scored as dense colo-

nies with a diameter of >0.5 mm after a 12–14-d incubation in 5% O<sub>2</sub>, 10% CO<sub>2</sub>, and 85% N<sub>2</sub>.

**Single-Cell Proliferation Assays.** Lin<sup>-</sup> or Lin<sup>-</sup>Sca-1<sup>+</sup> were seeded in Terasaki plates (Nunc, Kamstrup, Denmark) at a concentration of one cell per well in a volume of 20  $\mu$ l complete IMDM. Wells were scored for proliferation (>50 cells) after 12 d incubation at 37°C, 5% CO<sub>2</sub> in air.

**Cell Phenotyping.** 10<sup>4</sup> Lin<sup>-</sup>Sca-1<sup>+</sup> cells were plated in 1 ml of complete IMDM and incubated for 12 d with cytokines at 37°C, 5% CO<sub>2</sub> in air. Cell morphology was determined after Giemsa staining of cytospin preparations.

## Results

To investigate the ability of IL-12 to directly affect the proliferation of primitive murine bone marrow progenitors, single Lin<sup>-</sup>Sca-1<sup>+</sup> cells were plated into microtiter plates in the presence of purified rMuIL-12 in conjunction with HGFs previously shown to affect stem cell growth (Table 1). As shown by others (5, 6), the only single HGF capable of inducing significant proliferation of the Lin<sup>-</sup>Sca-1<sup>+</sup> stem cells was IL-3. While IL-12 did not stimulate any proliferation alone, it synergized with a number of the HGFs investigated (Table 1). In particular, the frequency of Lin<sup>-</sup>Sca-1<sup>+</sup> cells responding to SCF with colony formation was enhanced from 1:100 to 1:12, representing an 8.3-fold increase in responding cells. In comparison, IL-1, a well-characterized synergistic factor, enhanced SCF-induced colony formation 267% (Table 1), while SCF combined with IL-3, GM-CSF, G-CSF, and CSF-1 resulted in a synergistic increase in colony numbers of 296, 547, 1145, and 342%, respectively (not shown). The IL-12-induced synergy on SCF-stimulated proliferation of Lin<sup>-</sup>Sca-1<sup>+</sup> stem cells was concentration dependent, with an ED<sub>50</sub> between 2 and 20 ng/ml, and maximum stimulation observed at 20–200 ng/ml (Fig. 1). A neutralizing anti-mouse IL-12 mAb blocked the synergistic effect of IL-12, demonstrating that the observed synergistic effect was due to IL-12 and not possible contaminants (Table 1). In addition, G-CSF, which alone did not induce any proliferation of Lin<sup>-</sup>Sca-1<sup>+</sup> cells, stimulated 1:60 Lin<sup>-</sup>Sca-1<sup>+</sup> cells to colony formation when cocultured with IL-12, while IL-12 enhanced CSF-1- and IL-3-stimulated colony formation 150 and 67%, respectively (Table 1). GM-CSF-stimulated colony formation of Lin<sup>-</sup>Sca-1<sup>+</sup> cells was, however, not significantly stimulated by IL-12, and in contrast to its ability to synergize with myeloid HGFs, we found no evidence that IL-12 could synergize with lymphoid growth factors, such as IL-2, IL-4, or IL-7, to stimulate lymphopoiesis from Lin<sup>-</sup>Sca-1<sup>+</sup> stem cells (Table 1). Finally, IL-12 synergistically interacted with several two-factor cytokine combinations to increase colony numbers (Table 1).

To determine whether the increased frequency of colonies formed in response to IL-12 was due to an increase in the size of responding clones from clusters to colony size and/or the result of an absolute increase in total responding progenitors, clones were scored based on the degree of cell proliferation (Table 2). These experiments demonstrated that IL-12 increased the number of responding progenitor cells as well

**Table 1.** Effects of IL-12 on Proliferation of Single Lin<sup>-</sup>Sca-1<sup>+</sup> Bone Marrow Cells

Cytokine	Colonies per 1,000 cells		Fold increase
	- IL-12	+ IL-12	
-	0	0	7
G-CSF	0	17	NA*
CSF-1	13	33	2.5†
GM-CSF	10	13	1.3
IL-3	40	67	1.7*
SCF	10	83	8.3*
IL-1	0	0	1
IL-2	0	0	1
IL-4	0	0	1
IL-7	0	0	1
SCF + IL-1	37	99	2.7*
G-CSF + CSF-1	51	84	1.7†
G-CSF + GM-CSF	30	44	1.5
SCF + anti-IL-12	10	13	1.3
SCF + G-CSF	124	ND	NA

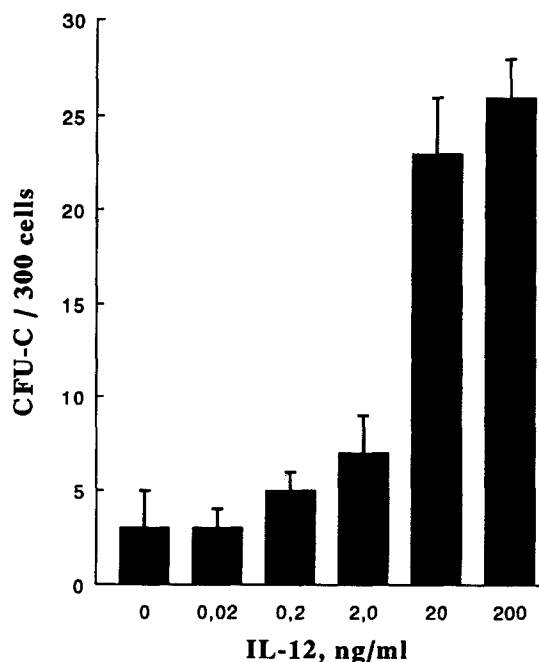
Bone marrow cells were obtained from femurs of 20–40 C57BL mice and Lin<sup>-</sup>Sca-1<sup>+</sup> cells separated as described in Materials and Methods. Lin<sup>-</sup>Sca-1<sup>+</sup> cells were plated in microtiter wells at a concentration of one cell per well in 20  $\mu$ l IMDM supplemented with 20% FCS and predetermined optimal concentrations of purified recombinant growth factors as described in Materials and Methods. Wells were scored for cell growth (>50 cells) after a 12–14-d incubation at 37°C, 5% CO<sub>2</sub> in air in the presence or absence of purified rMuIL-12 (50 ng/ml). Rat anti-murine IL-12 mAb was used at 50  $\mu$ g/ml, and an isotype-matched control had no effect on IL-12-induced proliferation. The results represent the mean of four separate experiments, with a minimum of 1,200 wells scored per group. A two-tailed binominal test was performed comparing - IL-12 vs. + IL-12. NA, not applicable.

\*  $p \leq 0.001$ .

†  $p \leq 0.01$ .

as the size of the colonies formed. In particular, IL-12 potentially enhanced the number of big colonies (covering >25% of the well) in combination with SCF, resulting in the formation of 0 and 43 such colonies (per 1,000 cells) in the absence and presence of IL-12, respectively (Table 2).

Although the Lin<sup>-</sup>Sca-1<sup>+</sup> cells contain the most immature stem cell population isolated to date, some intrinsic heterogeneity has been demonstrated in vitro as well as in vivo (13, 20). Therefore, we next investigated the ability of IL-12 to stimulate the HPP-CFCs within the Lin<sup>-</sup>Sca-1<sup>+</sup> population, since the HPP-CFC is among the most primitive stem cells shown to proliferate in vitro (19, 21, 22). While few or no HPP-CFCs were stimulated by the investigated growth factor combinations in the absence of IL-12, addition of IL-12 (50 ng/ml) resulted in a potent enhancement of colony for-



**Figure 1.** Dose-response of IL-12-induced synergy on SCF-supported proliferation of Lin<sup>-</sup>Sca-1<sup>+</sup> bone marrow cells. Lin<sup>-</sup>Sca-1<sup>+</sup> cells were isolated as described in Materials and Methods and plated at a density of one cell per well in microtiter plates in medium supplemented with rrSCF 100 ng/ml, and exposed to increasing concentrations of rMuIL-12 as indicated. Wells were scored for colony formation (>50 cells) after a 12–14-d incubation at 37°C in 5% CO<sub>2</sub> in air. Results represent mean (SEM) of four separate experiments.

**Table 2.** Degree of Lin<sup>-</sup>Sca-1<sup>+</sup> Cell Proliferation in the Absence and Presence of IL-12

Growth factors	Degree of cell proliferation*					Total no. clones per 1,000 cells
	1	2	3	4	5	
G-CSF	2	0	0	0	0	2
G-CSF + IL-12	4	9	5	0	0	18
CSF-1	4	7	4	1	0	16
CSF-1 + IL-12	8	10	16	5	0	39
SCF	8	8	2	0	0	18
SCF + IL-12	12	15	24	19	24	94

Lin<sup>-</sup>Sca-1<sup>+</sup> cells, separated as described in Materials and Methods, were plated in microtiter plates at a concentration of one cell per well in 20  $\mu$ l IMDM supplemented with 20% FCS and predetermined optimal concentrations of growth factors. Wells were scored for cell growth after a 12-d incubation at 37°C, 5% CO<sub>2</sub> in air in the presence or absence of rMuIL-12 (50 ng/ml).

\* Scoring criteria: 1, 10–50 cells; 2, 50 cells to 10% of well covered by cells; 3, cells covering 10–25% of well; 4, 25–50% of well covered; 5, >50% of well covered by cells. The results presented represent one of three similar experiments.

mation of HPP-CFCs (Table 3), confirming that IL-12 is a synergistic factor for one of the earliest stem cells measurable in vitro.

To determine whether IL-12 also could affect the differentiation potential of SCF, Lin<sup>-</sup>Sca-1<sup>+</sup> cells were grown in SCF in the presence or absence of IL-12 (50 ng/ml) for 14 d, and cytopsin preparations were analyzed morphologically. Only granulocytes and macrophages were observed in SCF-stimulated cultures, and while IL-12 dramatically enhanced the total number of cells, it did not affect the relative content of granulocytes and macrophages (Table 4). These findings were further supported by isolation of individual Lin<sup>-</sup>Sca-1<sup>+</sup> clones, demonstrating that the relative contribution of granulocyte, macrophage, and mixed (granulocyte/macrophage) colonies was not influenced by IL-12 treatment (Table 5). Similarly, IL-12 did not affect the differentiation of cultures stimulated by G-CSF + GM-CSF or G-CSF + CSF-1 (not shown). Thus, the synergistic effects of IL-12 on Lin<sup>-</sup>Sca-1<sup>+</sup> cell proliferation do not affect the ability of the cooperating myeloid growth factors to stimulate myelopoietic differentiation.

We next examined whether IL-12 also could affect the growth of more committed myeloid progenitors. While IL-12 had no stimulatory activity alone, it enhanced the colony formation of both low density and Lin<sup>-</sup> bone marrow cells in response to SCF, G-CSF, CSF-1, and GM-CSF, while IL-3-stimulated colony formation was not affected by IL-12 treatment (Table 6). The stimulatory effects of rMuIL-12 on Lin<sup>-</sup> progenitors were directly mediated, since a similar pattern of stimulation could be observed when Lin<sup>-</sup> cells were plated individually (not shown). Similar results were observed on Lin<sup>-</sup>Sca-1<sup>-</sup> progenitors (not shown). However, the syn-

**Table 3.** *The Effect of IL-12 on Lin<sup>-</sup>Sca-1<sup>+</sup> HPP-CFC*

Growth factors	HPP-CFC	
	- IL-12	+ IL-12
SCF	0	6(1)*
SCF + IL-1	0	14(3)*
G-CSF + CSF-1	10(2)	24(4)*
G-CSF + GM-CSF	2(1)	8(2)†
SCF + IL-6	0	6(1)*

Lin<sup>-</sup>Sca-1<sup>+</sup> cells were separated as described in Table 1 and plated into a double-layer agar culture as described in Materials and Methods. The top layer contained 1,000 Lin<sup>-</sup>Sca-1<sup>+</sup> cells in 0.3% sea plaque agarose. Growth factors were incorporated into the underlayer at predetermined optimal concentrations (Materials and Methods). Cultures were scored for HPP-CFC growth (tight colonies with a diameter >0.5 mm) after a 14-d incubation in 5% O<sub>2</sub>, 10% CO<sub>2</sub>, and 85% N<sub>2</sub>, at 37°C in the absence or presence of rMuIL-12 (50 mg/ml). The results represent the mean (SEM) of triplicate determinations, and are representative of four separate experiments. A two-tailed binominal test was performed, comparing - IL-12 vs. + IL-12.

\*  $p \leq 0.001$ .

†  $p \leq 0.05$ .

**Table 4.** *The Effect of IL-12 on Differentiation of SCF-stimulated Lin<sup>-</sup>Sca-1<sup>+</sup> Bone Marrow Cells in Bulk Liquid Culture*

Growth factors	Cells/ml	Percent blasts	Percent granulocytes	Percent macrophages
SCF	$8.0 \times 10^4$	3(2)	83(5)	14(6)
SCF + IL-12	$180 \times 10^4$	4(1)	87(8)	9(8)

Lin<sup>-</sup>Sca-1<sup>+</sup> cells were plated in liquid culture at 10,000 cells/ml in IMDM with 20% FCS and rrSCF 100 ng/ml in the presence or absence of purified rMuIL-12 (50 ng/ml). Cell morphology was determined after Giemsa staining (Sigma Chemical Co.) of cytopsin preparations from bulk liquid culture. Results represent mean (SEM) of three separate experiments.

ergistic effects of IL-12 observed on these progenitor cell populations were much less pronounced than on the Lin<sup>-</sup>Sca-1<sup>+</sup> stem cells. Thus, in addition to its previously demonstrated effects on mature B and T cells, IL-12 is a potent synergistic factor for stem cells as well as committed myeloid progenitor cells in the bone marrow.

## Discussion

Intensive research is still being dedicated to the isolation and characterization of growth factors capable of stimulating stem cells in bone marrow, since no defined growth factor combinations can stimulate these stem cells to proliferate with a similar potency as observed when cocultured with a stroma-feeder layer (23). Although there are probably multiple reasons for this difference, involving for instance direct stem cell-stroma cell interactions, it is likely that the suboptimal proliferation of stem cells observed in response to the known HGFs is also partially due to the existence of additional stem cell growth factors yet not identified. In the present study we demonstrate that IL-12 is a growth factor with stem cell activity. The synergistic effects observed were directly medi-

**Table 5.** *The Effect of IL-12 on Differentiation of SCF-stimulated Individual Lin<sup>-</sup>Sca-1<sup>+</sup> Bone Marrow Cell Colonies*

Growth factors	Colony morphology		
	CFU-G	CFU-M	CFU-GM
		%	
SCF	78	4	18
SCF + IL-12	84	2	14

Lin<sup>-</sup>Sca-1<sup>+</sup> cells were plated in liquid culture at one cell per well in IMDM with 20% FCS and rrSCF 100 ng/ml in the presence or absence of purified rMuIL-12 (50 ng/ml). Cell morphology was determined after Giemsa staining (Sigma Chemical Co.) of cytopsin preparations of individual colonies. For both groups, a total of 60 colonies were sampled from three separate experiments.

**Table 6.** The Effect of IL-12 on Colony Formation of Unfractionated, Low Density, and Lin<sup>-</sup> Bone Marrow Cells

CSF	IL-12	Unfractionated bone marrow	No. CFU-C	
			LDBM	Lin <sup>-</sup>
-	-	0	0	0
-	+	0	0	0
G-CSF	-	ND	5(1)	8(1)
G-CSF	+	ND	10(0)	17(1)
CSF-1	-	21(5)	26(3)	88(4)
CSF-1	+	30(3)	35(2)	122(12)
GM-CSF	-	19(6)	23(2)	44(5)
GM-CSF	+	27(3)	33(2)	61(6)
IL-3	-	ND	16(2)	69(7)
IL-3	+	ND	15(3)	66(3)
SCF	-	ND	ND	34(5)
SCF	+	ND	ND	46(7)

Low density and Lin<sup>-</sup> bone marrow cells were separated and plated as described in Materials and Methods at a density of  $7.5 \times 10^4$  (unfractionated),  $5 \times 10^4$  (low density), and  $2 \times 10^4$  (Lin<sup>-</sup>) cells/ml in IMDM containing 20% FCS and 0.3% sea plaque agarose supplemented with predetermined optimal concentrations of growth factors. After a 7-d incubation (37°C, 5% CO<sub>2</sub>) in the absence or presence of rMuIL-12 (50 ng/ml), cultures were scored for colony growth >50 cells. Results represent the mean (SEM) of three separate experiments.

ated on the hematopoietic stem cell, since single-cell cloning experiments demonstrated the same pattern of IL-12 response.

The most potent synergy of IL-12 was observed in combination with SCF, a HGF playing an important role in maintaining viability and inducing proliferation of the most immature stem cells (4-6, 10, 11). IL-12 increased the frequency of responding progenitor cells as well as the size of the colonies. The fact that IL-12 most potently stimulated the formation of large colonies in single-cell cultures, and more specifically the HPP-CFC, suggests that IL-12 acts preferentially on immature progenitor cells. Furthermore, the synergistic effect of IL-12 on stem cell proliferation is very potent, since only the synergy observed between G-CSF and SCF was stronger than between IL-12 and SCF. In addition IL-12 enhanced the proliferation of more committed myeloid progenitor cells as well. Since IL-12 only enhanced the proliferation of Lin<sup>-</sup>Sca-1<sup>+</sup> stem cells when combined with myeloid growth factors, it was not surprising that the progeny produced were exclusively of myeloid origin. However, since the Lin<sup>-</sup>Sca-1<sup>+</sup> cells seem to be able to form only myeloid colonies under defined in vitro conditions, while under in vivo conditions they can form lymphoid progeny as well, we can not exclude that IL-12 might (under optimal conditions) induce lymphopoiesis from hematopoietic stem cells as well.

It has been proposed that CLMF should be assigned the name IL-12, due to its pleiotropic effects on mature lymphoid cells (17). The present study further justifies this designation, demonstrating that IL-12 is a cytokine with pleiotropic effects also on the immature stem cells and committed myeloid progenitors.

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