Interleukin 13: Novel Role in Direct Regulation of Proliferation and Differentiation of Primitive Hematopoietic Progenitor Cells

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Summary

The recently cloned interleukin 13 (IL-13) shares most investigated biological activities on B lymphocytes and monocytes with IL-4. In this study we investigated for the first time the potential role of IL-13 in the regulation of the growth of hematopoietic progenitor cells. IL-13 enhanced stem cell factor (SCF)–induced proliferation of $Lin^-Sca\text{-}1^+$ bone marrow progenitor cells more potently than IL-4. The effect of IL-13 was purely synergistic, since IL-13 alone stimulated no colony formation. Single cell experiments suggested that the synergistic effect of IL-13 on $Lin^-Sca\text{-}1^+$ progenitors was directly mediated. In contrast, IL-13 had no synergistic activity on SCF-induced proliferation of the more mature $Lin^-Sca\text{-}1^-$ progenitor cells. Thus, the cloning frequency in response to SCF + IL-13 was at least 20-fold higher in the $Lin^-Sca\text{-}1^+$ than the $Lin^-Sca\text{-}1^-$ progenitor cell population. Furthermore, IL-13 but not IL-4 synergistically enhanced colony formation of $Lin^-Sca\text{-}1^+$ progenitors in response to granulocyte/macrophage colony-stimulating factor (GM-CSF) (threefold), whereas both IL-4 and IL-13 enhanced G-CSF–induced colony formation (threefold), and neither of the two significantly affected CSF-1 and IL-3–induced proliferation. Finally, whereas stimulation of $Lin^-Sca\text{-}1^+$ progenitors by SCF + G-CSF resulted in the formation of 90% granulocytes, the addition of IL-13 resulted in the production of macrophages exclusively. This novel effect on differentiation was directly mediated, shared with IL-4, and could not be observed on $Lin^-Sca\text{-}1^-$ progenitor cells. Collectively, these findings indicate a novel role of IL-13 in early myelopoiesis, partially overlapping but also different from that of IL-4.

Hematopoiesis is a lifelong process in which short-lived mature hematopoietic cells are continuously replenished by a pool of primitive pluripotent hematopoietic stem cells (1–3). It seems that steady state hematopoiesis, where most stem cells reside in quiescence, is regulated at least in part by the opposing actions of stimulatory and inhibitory cytokines. CSFs, which include G-CSF, M-CSF or CSF-1, GM-CSF, and IL-3 or multi-CSF, are a unique family of hematopoietic growth factors (HGFs)[1] in that they can promote the in vitro proliferation and granulocyte-macrophage differentiation of hematopoietic progenitor cells in the absence of other HGFs (1, 4). In contrast, a number of other HGFs, including multiple interleukins and stem cell factor (SCF, also called kit ligand, steel factor, or mast cell growth factor) have little or no effect on the growth of hematopoietic progenitor cells when acting alone, but can synergistically enhance their proliferative response to the CSFs and/or SCF (3, 5–14). Synergistic HGFs are of particular importance in the regulation of primitive hematopoietic progenitor cells, since they, in contrast to more mature progenitors, can only be triggered to proliferate optimally as a result of synergy between multiple HGFs (13–16).

IL-4, is one of many cytokines produced by activated T cells. It stimulates B cells (17, 18), T cells (19, 20), and mast cells (19, 20). Furthermore, it has been demonstrated to bifunctionally affect the in vitro growth of hematopoietic progenitor cells (21, 22). IL-13 is another recently cloned T cell–derived cytokine that shows homology with IL-4 (23, 24). IL-4 and IL-13 appear to have a common signaling receptor subunit, whereas they have separate ligand-binding subunits (25, 26). This probably explains why IL-4 and IL-13 show overlapping but also distinctly different patterns of biological activities on investigated cell types (23–27). Thus, IL-13 but not IL-4 can stimulate IFN-γ synthesis by large

[1] *Abbreviations used in this paper:* HGF, hematopoietic growth factor; rHu, recombinant human; rMu, recombinant murine; rr, recombinant rat; SCF, stem cell factor.

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granular lymphocytes (23), whereas PHA-activated PBMC proliferate in response to IL-4 but not IL-13 (25).

Since the role of IL-13 in hematopoiesis has not yet been established, we investigated whether IL-13 could affect the growth of mature as well as primitive murine hematopoietic progenitor cells, and to what extent its effects overlap with those of IL-4.

Materials and Methods

HGFs. Recombinant human (rHu)IL-13 was purified from culture supernatants of stably transfected CHO cell lines as previously described (23). Three different lots of rHuIL-13 (1PE, 4PE, and 6PE) were used in the present studies. Purified recombinant murine (rMu)IL-3 and IL-4 were purchased from Peprotech Inc. (Rocky Hill, NJ). Purified rHuG-CSF, rMuGM-CSF, and recombinant rat (rr)SCF were generously supplied by Drs. Ian K. McNiece, Thomas C. Boone, and Keith E. Langley (Amgen Corp., Thousand Oaks, CA). rHuCSF-1 was kindly provided by Dr. Michael Geier (Cetus Corp., Emeryville, CA). rHuIL-11 was a generous gift from Genetics Institute (Cambridge, MA), purified rHuIL-1α was supplied by Hoffmann-La Roche (Basel, Switzerland). Unless otherwise indicated, all growth factors were used at predetermined optimal concentrations: rMuGM-CSF, 20 ng/ml; rHuG-CSF, 50 ng/ml; rMuIL-3, 20 ng/ml; rHuCSF-1, 50 ng/ml; rrSCF, 100 ng/ml; rHuIL-1α, 20 ng/ml; and rHuIL-11, 50 ng/ml.

Isolation of Lin$^-$Sca-1$^+$ and Lin$^-$Sca-1$^-$ Bone Marrow Cells. Lin$^-$ bone marrow cells were isolated from femurs of normal C57BL/6 mice (5–8-wk-old), according to a previously described protocol (2). 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), 100 U/ml penicillin, and 3 mg/ml L-glutamine (complete IMDM). 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; Beckton 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 (Dynal, Oslo, Norway) were added at a cell/bead ratio of 1:20, and incubated at 4°C for 30 min. Labeled (Lin$^+$) cells were removed by a magnetic particle concentrator (Dynal), and Lin$^-$ cells recovered from the supernatant. Lin$^-$Sca-1$^+$ cells were isolated as described by others (2, 28). Briefly, 4–6 × 10^7 Lin$^-$ cells were resuspended per milliliter of complete IMDM and incubated for 30 min on ice with either FITC-conjugated rat anti-mouse SCA-1 antibody (cone E13167-7; PharMingen) or an isotype-matched control antibody. The cells were washed twice, and Lin$^-$Sca-1$^+$ cells sorted on a cell sorter (Epics Elite; 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$^-$ 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 was collected through a 488-nm band pass filter and FITC fluorescence was collected through a 488-nm-long pass filter and a 525-nm band pass filter. The final recovery of Lin$^-$Sca-1$^+$ cells was 0.05–0.1% of the unfractionated bone marrow. In some experiments, the remaining Lin$^-$Sca-1$^-$ cells were collected as well.

Single Cell Proliferation Assay. Lin$^-$Sca-1$^+$ or Lin$^-$Sca-1$^-$ cells were seeded in microtiter plates (Nunc, Kamstrup, Denmark) at a concentration of one cell per well in a volume of 20 μl complete IMDM. Wells were scored for colony growth (>50 cells) and clusters (10–50 cells) after 12 d of incubation at 37°C and 5% CO_2 in air.

Cell Morphology. Lin$^-$Sca-1$^+$ or Lin$^-$Sca-1$^-$ cells were plated in complete IMDM and incubated for 12–14 d with predetermined optimal concentrations of cytokines at 37°C and 5% CO_2 in air. Cell morphology was determined following Giemsa staining of cytospin preparations.

Results

IL-13 Enhances SCF-induced Colony Formation of Single Lin$^-$Sca-1$^+$ Progenitor but Not Lin$^-$Sca-1$^-$ Progenitor Cells. The infrequent Lin$^-$Sca-1$^+$ cells in murine bone marrow have been demonstrated to be highly enriched in primitive hematopoietic progenitors. Thus, in agreement with others (2, 28), we have observed that as few as 100 of these cells can rescue 50% of lethally irradiated mice (Veiby, O. P., unpublished observations). In contrast, 3–4 × 10^4 unfractionated bone marrow cells are required to give the same degree of protection (29). Furthermore, Lin$^-$Sca-1$^+$ cells can efficiently long-term reconstitute all cell lineages of the blood (2, 28), and proliferate in vitro in response to defined HGFs (13, 14, 30, 31). In this study, Lin$^-$Sca-1$^+$ cells (300 per group) were plated individually to compare the direct effects of IL-13 and IL-4 on primitive bone marrow progenitor cells (Fig. 1). Neither rHuIL-13 (1–500 ng/ml) nor rMu IL-4 (1–500 ng/ml) alone stimulated the proliferation of Lin$^-$Sca-1$^+$ cells cultured individually (Fig. 1). Whereas G-CSF alone stimulated the formation of only two colonies of Lin$^-$Sca-1$^+$ cells it synergized with IL-13 as well as IL-4, resulting in the formation of 10 and 9 colonies, respectively.

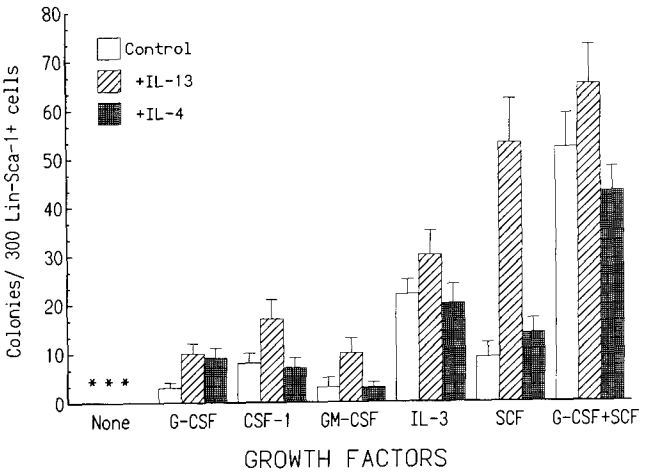


Figure 1. Effects of IL-13 and IL-4 on the proliferation of single Lin$^-$Sca-1$^+$ bone marrow progenitors. Lin$^-$Sca-1$^+$ bone marrow cells were isolated as described in Materials and Methods and plated at one cell per well in 20 μl complete IMDM supplemented with predetermined optimal concentrations of cytokines as indicated. Wells were scored for colonies (>50 cells) after 12–14 d of incubation at 37°C and 5% CO_2 in air in the absence or presence of IL-13 (500 ng/ml) and IL-4 (100 ng/ml). The results represent the mean (SEM) of four separate experiments, with a minimum of 1,200 wells scored per group. (*) No colonies were observed in the absence of growth factors or in the presence of IL-13 and IL-4 alone.

Whereas IL-4 failed to synergize with CSF-1 and GM-CSF, IL-13 enhanced CSF-1 and GM-CSF–induced colony formation two- and three-fold, respectively (Fig. 1). In contrast, neither IL-13 nor IL-4 significantly affected the number of colonies formed by Lin$^-$Sca-1$^+$ progenitors in response to IL-3.

In agreement with previously published data SCF (100 ng/ml) alone stimulated some colony formation of single Lin$^-$Sca-1$^+$ cells (13, 14), and IL-4 (at 1–500 ng/ml) only marginally increased the number of SCF-responsive progenitors (from 9 to 14 colonies; Fig. 1). In contrast, the addition of IL-13 (500 ng/ml) resulted in a sixfold increase in colony formation (from 9 to 53 colonies; Fig. 1). In comparison, SCF + G-CSF, a very potent proliferation stimulus for Lin$^-$Sca-1$^+$ progenitor cells (13, 14), stimulated the same number of colonies as SCF + IL-13, and the addition of IL-13 did not significantly enhance the SCF + G-CSF response (Fig. 1). The synergistic effect of IL-13 on SCF-stimulated proliferation occurred in a concentration-dependent fashion with an ED_{50} of 50–100 ng/ml, and maximum effect observed at 500 ng/ml (data not shown). The results described above were obtained using two different lots of purified rHuIL-13 (1PE and 4PE). In more recent experiments, using a third lot of rHuIL-13 (6PE), we observed the same magnitude of synergy with G-CSF or GM-CSF, but no significant synergy with CSF-1, and much less synergy with SCF (data not shown). Specifically, the 6PE lot of IL-13 enhanced SCF-induced colony formation 2.5-fold as compared with 6-fold with the two first lots of IL-13, and 1.5-fold with IL-4. In addition, whereas the first two lots of rHuIL-13 also enhanced the size of the colonies formed in response to SCF, the 6PE lot had no such effect on the size of the SCF-responsiveness clones (data not shown).

Table 1. *Effect of IL-13 and IL-4 on the Myeloid Differentation of Lin$^-$Sca-1$^+$ Bone Marrow Cells*

Growth factors	Percent blasts	Percent granulocytes	Percent macrophages
SCF	4 (1)	90 (3)	6 (2)
SCF + IL-13	4 (1)	89 (3)	8 (3)
SCF + IL-4	4 (2)	85 (7)	11 (6)
SCF + G-CSF	2 (1)	89 (3)	8 (4)
SCF + G-CSF + IL-13	0 (0)	2 (1)	98 (1)
SCF + G-CSF + IL-4	0 (1)	13 (5)	87 (6)
SCF + IL-11	26 (4)	58 (7)	16 (7)
SCF + IL-11 + IL-13	8 (5)	9 (5)	81 (11)
SCF + IL-11 + IL-4	5 (3)	12 (6)	83 (10)

250 Lin$^-$Sca-1$^+$ cells were plated in 500 μl complete IMDM in the presence of predetermined optimal concentrations of growth factors (Materials and Methods) as indicated, and in the absence or presence of IL-13 (100 ng/ml) or IL-4 (20 ng/ml). Cytospin preparations were prepared and stained with Giemsa staining (Sigma Chemical Co., St. Louis, MO) after 14 d of incubation at 37°C and 5% CO_2 in air. Cell morphology was determined on at least 100 cells per group in each experiment. Results represent the mean (SEM) of four separate experiments.

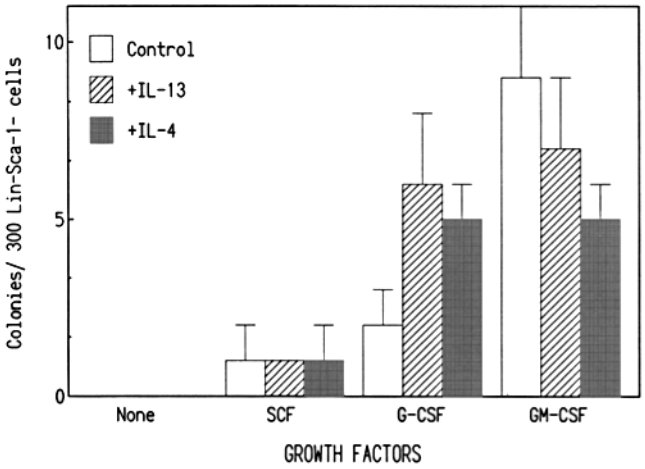
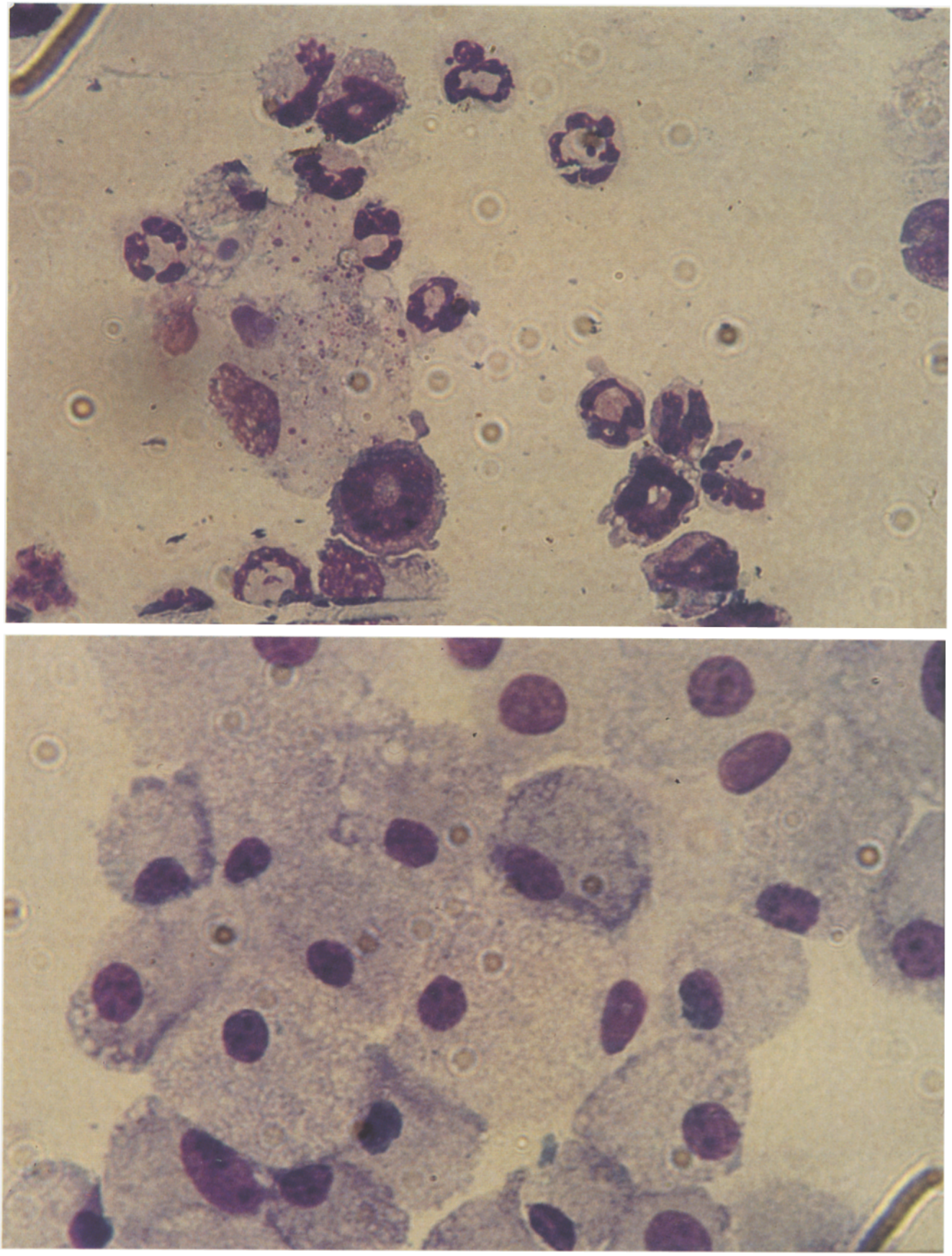


Figure 2. The effects of IL-13 and IL-4 on the proliferation of single Lin$^-$Sca-1$^-$ bone marrow progenitors. Lin$^-$Sca-1$^-$ bone marrow cells were isolated as described in Materials and Methods and cultured at one cell per well in 20 μl complete IMDM in the absence of growth factors or supplemented with predetermined optimal concentrations of SCF, G-CSF, and GM-CSF. A total of 300 wells were cultured per group and colony growth (>50 cells) was determined after 7 d of incubation at 37°C and 5% CO_2 in air in the absence or presence of IL-13 (500 ng/ml) or IL-4 (100 ng/ml). Results represent the mean (SEM) of three separate experiments. No colony formation was observed in the absence of growth factors or in the presence of IL-13 or IL-4 alone.

The Lin$^-$Sca-1$^-$ bone marrow cells which are depleted of the most primitive hematopoietic progenitors (2, 13, 28, 30), and which thus represent more mature progenitors, were next explored for the potential effects of IL-13. In accordance with others (30) these colonies appeared and disappeared earlier than the Lin$^-$Sca-1$^+$ progenitors. Colonies were therefore scored after 7 d (Fig. 2) as well as after 14 d of incubation (data not shown), and although the colony numbers were slightly lower on day 14, the effects of IL-13 and IL-4 were similar on days 7 and 14 (data not shown). As previously demonstrated (13), few or no colonies were formed when Lin$^-$Sca-1$^-$ progenitors were plated individually and stimulated by SCF or G-CSF alone (Fig. 2). In contrast to the ability of IL-13 to enhance SCF-induced proliferation of Lin$^-$Sca-1$^+$ progenitor cells, IL-13 had no effect on SCF-stimulated colony formation of Lin$^-$Sca-1$^-$ progenitors (Fig. 2). However, both IL-4 and IL-13 synergistically enhanced G-CSF–stimulated colony formation of Lin$^-$Sca-1$^-$ progenitors, increasing the cloning frequency two- to three-fold. In agreement with previous studies (31–33), IL-4 inhibited GM-CSF–stimulated colony formation of single Lin$^-$Sca-1$^-$ progenitors (from nine to five colonies; Fig. 2). In contrast to its potent synergistic effect on GM-CSF–stimulated proliferation of Lin$^-$Sca-1$^+$ progenitors, IL-13 had no effect or slightly inhibited GM-CSF–stimulated colony formation of Lin$^-$Sca-1$^-$ progenitors (Fig. 2). The effects of IL-13 on colony formation of Lin$^-$Sca-1$^-$ progenitors did



not differ between the three rHuIL-13 lots tested (data not shown). Thus, IL-13 differentially regulates the proliferation of primitive and more mature hematopoietic progenitor cells.

IL-13 Blocks SCF + G-CSF–induced Granulocyte Differentiation of Lin⁻Sca-1⁺ Progeny, and Stimulates Exclusively Macrophage Production. Since IL-13 potently enhanced SCF-induced proliferation of Lin⁻Sca-1⁺ progenitors, we next asked whether IL-13 also could affect the differentiation of SCF-stimulated Lin⁻Sca-1⁺ progeny cultured in liquid culture. As previously demonstrated (12, 13), SCF alone stimulated the production of mature granulocytes almost exclusively (90%; Table 1). In addition, a low number of macrophages (6%) and blasts (4%) was observed. Whereas IL-13 enhanced SCF-induced cell production (data not shown), it did not significantly affect the relative proportion of granulocytes, macrophages, or blasts (Table 1). Next, SCF was combined with G-CSF, a two-factor combination demonstrated to potently stimulate granulopoiesis of primitive hematopoietic progenitors (12, 34). The addition of IL-13 did not significantly affect the number of cells produced in response to SCF + G-CSF (data not shown). However, an almost complete switch from granulocyte to macrophage production was observed in response to all three rHuIL-13 lots (Table 1 and Fig. 3). The switch in differentiation was concentration dependent (Fig. 4), and was observed at lower concentrations of IL-13 than the synergistic effect on SCF-induced proliferation (ED_{50} of 1–10 ng/ml and 50–100 ng/ml, respectively).

SCF + IL-11 is another growth factor combination demonstrated to potently stimulate granulocyte/macrophage production of primitive progenitors (8, 35). As much as 26% of SCF + IL-11–stimulated Lin⁻Sca-1⁺ progeny observed in culture after 14 d had the appearance of blasts (Table 1). Whereas IL-13 did not significantly affect the total cell production in response to SCF + IL-11 (data not shown), it reduced the proportion of granulocytes from 58 to 9%, blasts from 26 to 8%, with a concomitant increase in macrophages (from 16 to 81%; Table 1). IL-13 also resulted in a dramatic shift toward macrophage production when combined with other potent granulocyte stimuli, such as SCF + IL-1, SCF + IL-6, or SCF + IL-12 (data not shown). Finally, IL-4 showed comparable effects to IL-13 on the differentiation of Lin⁻Sca-1⁺ progeny (Table 1). Thus, both IL-13 and IL-4 can potently inhibit granulocyte differentiation and stimulate macrophage differentiation of Lin⁻Sca-1⁺ progeny.

To exclude the possibility of the stimulatory effect of IL-13 on SCF + G-CSF–induced macrophage production being indirectly mediated through potentially contaminating accessory cells, the myeloid differentiation of Lin⁻Sca-1⁺ progeny was also assessed by morphological examination of Lin⁻Sca-1⁺ clones cultured and examined individually (Table 2). These experiments were also performed to determine whether the observed increase in macrophage production was due to an absolute increase in progenitors producing exclusively macrophages (CFU-M). SCF + G-CSF–stimulated proliferation of CFU-G was completely blocked by IL-13 (100 ng/ml) and the number of CFU-GM inhibited by 80%, resulting in a more than eightfold increase in the number of CFU-M (Table 2). Furthermore, since the total number of colonies formed in response to SCF + G-CSF was not significantly affected by IL-13 (Table 2), these findings demonstrated a potent absolute increase in the number of Lin⁻Sca-1⁺ progenitors producing exclusively macrophages.

Table 2. *Effects of IL-13 on the Morphology of SCF + G-CSF–induced Colonies Derived from Single Lin⁻Sca-1⁺ Bone Marrow Progenitors*

Growth factors	Colonies/ 300 cells	Colony morphology		
		CFU-G	CFU-M	CFU-GM
		%		
SCF + G-CSF	62 (10)	49	8	43
SCF + G-CSF + IL-13	73 (15)	0	90	10

Lin⁻Sca-1⁺ cells were plated at one cell per well in complete IMDM as described in Materials and Methods, and supplemented with SCF (100 ng/ml) and G-CSF (50 ng/ml). Cultures were scored for colony growth after 14 d of incubation at 37°C and 5% CO_2, in the absence or presence of IL-13 (500 ng/ml). The number of colonies presented are the mean (SEM) from four separate experiments. Colony morphology was determined after Giemsa staining of cytospin preparations of individual colonies. For both groups a total of at least 60 colonies was sampled from four separate experiments.

To examine the effects of IL-13 on the differentiation of more mature progenitors, Lin⁻Sca-1⁻ bone marrow cells were next cultured in SCF + G-CSF in the presence or absence of IL-13. Since the peak production of mature myeloid cells from Lin⁻Sca-1⁻ cells was observed after 1 wk of incubation, and since most cells were dead by 2 wk (data not shown), cell morphology was examined on day 7 (Table 3). Furthermore, 20,000 SCF + G-CSF–stimulated Lin⁻Sca-1⁻ as opposed to 250 Lin⁻Sca-1⁺ cells were required to generate enough cells for cytospin preparations. Neither IL-13 (500 ng/ml) nor IL-4 (20 ng/ml) affected the relative production of granulocytes and macrophages of Lin⁻Sca-1⁻ progenitors in response to SCF + G-CSF (Table 3). Thus, IL-13 (and IL-4) preferentially stimulates macrophage production of primitive murine bone marrow progenitor cells.

Figure 3. The effects of IL-13 on differentiation of Lin⁻Sca-1⁺ progeny. 250 Lin⁻Sca-1⁺ cells were plated in 500 μl complete IMDM supplemented with G-CSF (50 ng/ml) and SCF (100 ng/ml), and cultured for 14 d in the absence (*A*) or presence (*B*) of IL-13 (100 ng/ml). Cytospin preparation were prepared and stained with Giemsa. ×1000.

Table 3. *The Effects of IL-13 and IL-4 on SCF + G-CSF–induced Myeloid Differentiation of Lin⁻Sca-1⁻ Progenitor Cells*

Growth factors	Percent blasts	Percent granulocytes	Percent macrophages
SCF + G-CSF	3 (2)	92 (5)	6 (4)
SCF + G-CSF + IL-13	6 (3)	87 (4)	7 (6)
SCF + G-CSF + IL-4	5 (3)	94 (3)	5 (3)

Lin⁻Sca-1⁻ bone marrow cells were separated as described in Materials and Methods and 20,000 cells were plated in 500 μl complete IMDM supplemented with SCF (100 ng/ml) and G-CSF (50 ng/ml) and cultured at 37°C and 5% CO_2 in air in the absence or presence of IL-13 (100 ng/ml) or IL-4 (20 ng/ml). After 7 d of incubation cell morphology was determined on 100 cells per group after Giemsa staining of cytospin preparations. Results represent the mean (SEM) of three separate experiments.

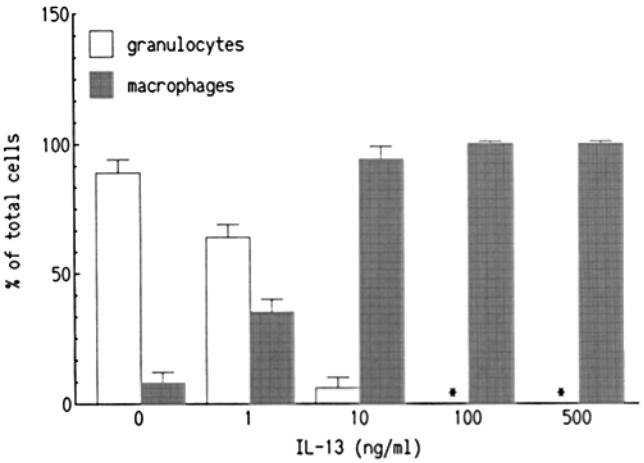


Figure 4. The dose–response of IL-13 on the differentiation of SCF + G-CSF–stimulated Lin⁻Sca-1⁺ progenitor cells. 250 Lin⁻Sca-1⁺ bone marrow cells were plated in 500 μl complete IMDM in the presence of SCF (100 ng/ml) and G-CSF (50 ng/ml), and exposed to increasing concentrations of IL-13 as indicated. After 14 d of incubation at 37°C and 5% CO_2 in air, cytospin preparations were stained with Giemsa and cell morphology determined on at least 100 cells per group in each experiment. Results presented represent the mean (SEM) of three separate experiments. (*) No granulocytes were observed in the presence of IL-13 100 and 500 ng/ml.

Discussion

In recent years, many synergistic growth factors capable of enhancing myelopoiesis of primitive hematopoietic progenitors have been identified and characterized, including IL-1, IL-6, IL-7, IL-11, and IL-12 (5–9). SCF, another synergistic HGF, has emerged as a key regulator of early hematopoiesis (10–14, 16, 35). SCF is unique in the sense that it can synergize with CSFs as well as other synergistic HGFs (10–14, 16, 35). Since it appears that primitive hematopoietic progenitors require multiple stimulatory signals to proliferate and differentiate, it remains important to identify HGFs that can synergize with SCF or CSFs to stimulate primitive hematopoietic progenitor cells. This study revealed that the newly cloned IL-13 can synergize with SCF as well as CSFs, increasing the number of colonies of SCF-stimulated Lin⁻Sca-1⁺ but not Lin⁻Sca-1⁻ bone marrow progenitors. This suggests that IL-13 in this regard preferentially acts on primitive progenitors. The fact that the synergistic effects of IL-13 were observed at the single cell level suggests that its effects are directly mediated on the progenitors, although autocrine mechanisms cannot be excluded. The present view that IL-13 can stimulate normal bone marrow progenitor cells is not surprising in light of the observation that the premyeloid cell line TF-1 also proliferates in response to IL-13 (24).

This is the first report demonstrating the effects of IL-13 on hematopoiesis. Of particular interest was the finding that IL-13 showed a pattern of synergistic activity different from that of IL-4, since these two cytokines show homology and even share a receptor subunit thought to be essential in signal transduction (23–26). The fact that IL-13 more potently enhanced SCF-induced proliferation of Lin⁻Sca-1⁺ progenitors than IL-4 could be explained by studies suggesting that the IL-4 and IL-13 receptor complexes use separate ligand binding proteins (25, 26). It is therefore possible that more SCF-responsive progenitors express the binding proteins of IL-13 than those of IL-4, or alternatively that the progenitors express higher levels of receptors for IL-13 than for IL-4. On the other hand, IL-13 and IL-4 were equally efficient in inducing a switch in SCF + G-CSF–induced differentiation of Lin⁻Sca-1⁺ progeny towards macrophages. Thus, this progenitor cell population would appear to have an expression of both IL-4 and IL-13 receptors above the threshold needed to elicit the effect on differentiation. An alternative explanation could be the existence of a separate low affinity receptor for IL-13 distinct from that of IL-4, which could signal the proliferative signal, whereas the common high affinity receptor would signal the differentiation of the Lin⁻Sca-1⁺ progenitors. In fact, such a distinct low affinity signal transducing receptor has been identified for IL-4 (36).

The hematopoietic effects of two original lots of purified rHuIL-13 (1PE and 4PE) used in the present studies, differed from a third lot in one important (quantitative) aspect, the magnitude of enhancement of SCF-stimulated proliferation of Lin⁻Sca-1⁺ progenitor cells. The other effects observed were similar between all three lots. The difference between the two previous (1PE and 4PE) and most recent (6PE) lots of rHuIL-13 is of particular interest, since all of them were purified according to the same procedure (23). Just recently we obtained a neutralizing antibody against IL-13 which appears to neutralize the effects of the new IL-13 lot (6PE), but only partially the enhancing effects of the two first IL-13 lots (1PE and 4PE) on SCF-stimulated proliferation, suggesting that the two first lots of IL-13 might contain contaminants capable of synergizing with SCF. If so, it might reflect the unique ability of SCF to synergize with numerous other cytokines to stimulate the growth of primitive progenitors (9–14). In addition, recent studies showing that very low levels of a cytokine are required to synergistically enhance the SCF response (37), in particular when multiple synergistic cytokines are acting in concert (like SCF and IL-13 here), suggest that such contaminating activities need only be present at very

low concentrations. This underscores the importance of confirming novel effects obtained with even highly purified recombinant cytokines by the use of neutralizing antibodies, in particular when looking at synergy with SCF.

Optimal enhancement of SCF-stimulated proliferation of $Lin^-Sca\text{-}1^+$ progenitors required as much as 500 ng/ml rHuIL-13, whereas the effects on differentiation of $Lin^-Sca\text{-}1^+$ progeny were observed at lower concentrations of rHuIL-13 (10 ng/ml). Other synergistic HGFs such as IL-6, IL-7, IL-12, and SCF are also required at high concentrations to optimally stimulate the growth of primitive hematopoietic progenitor cells (7, 9, 12–14). The optimal concentration of SCF or IL-6 for colony formation is, for instance, 100–500 ng/ml (12–14). The rHu IL-13 used in this study is 60% identical to MuIL-13 (23, 24), and MuIL-13 and HuIL-13 have similar specific activity on human cells (24). Whereas HuIL-13 is active on murine cells as well, it has recently been observed that HuIL-13 is required at approximately 100-fold higher concentrations than MuIL-13 to stimulate proliferation of the mouse B9 plasmacytoma cell line (38). Thus, rHuIL-13 might prove to be active on human hematopoietic progenitor cells at much lower concentrations than demonstrated here.

Whereas early acting synergistic HGFs such as IL-1, IL-6, IL-7, IL-11, and IL-12 can potently enhance myelopoiesis of $Lin^-Sca\text{-}1^+$ progenitor cells in combination with SCF and/or CSFs (13, 14), they appear to have little or no effect on the relative production of granulocytes and macrophages (7, 9, 16, 30, 35). In contrast, here we demonstrate a unique ability of IL-13 in combination with SCF + G-CSF to stimulate only macrophage production of $Lin^-Sca\text{-}1^+$ progenitors, whereas predominantly granulocytes were observed in the absence of IL-13. An IL-13–induced switch towards macrophage production was also observed with other two-factor combinations that included SCF. Single clone experiments demonstrated that this potent effect of IL-13 on differentiation was directly mediated, and due to an absolute increase in the number of CFU-M and a concomitant reduction in CFU-G. Not previously demonstrated, IL-4 also preferentially stimulated macrophage production from $Lin^-Sca\text{-}1^+$ progenitors. Since IL-4 also has been shown to stimulate granulocyte differentiation, and inhibit macrophage production (32, 33, 39) of more mature progenitors, it appears that IL-4 and IL-13 can bifunctionally modulate the myeloid differentiation of myeloid progenitors, depending on the maturity of the targeted progenitors as well on as the specific growth factors stimulating growth. In this regard, it is worth noticing that the effects of IL-13 and IL-4 on differentiation were not observed on $Lin^-Sca\text{-}1^-$ progenitor cells.

In conclusion, IL-13 and IL-4 show overlapping but also different patterns of regulation of the proliferation and differentiation of primitive murine hematopoietic progenitor cells.

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