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Research Article

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Enhancement of Human Granulopoiesis In Vitro by Biosynthetic Insulin-like Growth Factor I/Somatomedin C and Human Growth Hormone

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Abstract

The effect of biosynthetic recombinant insulin-like growth factor I/somatomedin C (IGF-I/Sm-C) and human growth hormone (hGH) on the in vitro growth and maturation of human marrow myeloid progenitors was investigated. Myeloid colony formation was maximally enhanced by 60 ng/ml IGF-I/Sm-C and by 250 ng/ml hGH, resulting in an increase in colony numbers of 41±7 and 38±4%, respectively ($P < 0.001$). Both peptides induced a 1.5–2.5-fold increase in the frequency of colonies composed of granulocytes alone, but did not alter the numbers of monocyte/macrophage or mixed granulocyte/macrophage colonies. IGF-I/Sm-C and hGH were also found to enhance myeloid maturation towards mature granulocytes in suspension cultures of human marrow cells. The effect of both peptides on human marrow granulopoiesis was similarly demonstrable in serum-free cultures stimulated with human recombinant granulocyte/macrophage colony-stimulating factor. Enhancement of human marrow granulopoiesis in vitro by hGH required the presence of marrow adherent cells and was abrogated by specific monoclonal antibodies directed against IGF-I/Sm-C receptors. The effect of hGH on marrow myeloid progenitors thus appears to be mediated by paracrine IGF-I/Sm-C.

Introduction

The insulin-like growth factors (IGFs)[1] are peptide growth factors sharing close structural homology with proinsulin (1), which stimulate mitogenesis and metabolic processes in a variety of cell types (2). In humans, two IGF peptides have been fully sequenced and cloned (3, 4). IGF-I/somatomedin C (IGF-I/Sm-C) is the presumed paracrine or autocrine mediator of growth hormone in peripheral tissues (5), and IGF-II, a less potent growth factor, is more insulin-like in its mode of action (6).

Membrane receptors for IGF-I/Sm-C have been detected on various mammalian cells (7–10), including human hemopoietic cells such as erythrocytes (11) and myeloid leukemic cells (12). IGF-I/Sm-C has recently been shown to enhance colony formation in vitro by human primitive and relatively mature marrow erythroid progenitors (13), an effect also known to be induced by human growth hormone (hGH) (14, 15).

The purpose of the present study was to investigate the effect of biosynthetic IGF-I/Sm-C on the growth and maturation of cultured human marrow myeloid progenitors (granulocyte/macrophage CFU [GM-CFU]) and to compare it to the effect of biosynthetic hGH. The possible role of IGF-I/Sm-C as a mediator of hGH was examined by the use of a monoclonal antibody directed against IGF-I/Sm-C receptors.

Methods

Preparation of marrow cells. Human bone marrow, obtained by informed consent from hematologically normal patients undergoing open-heart surgery, was aspirated into heparinized syringes. Marrow aspirates were diluted threefold in HBSS and subjected to Ficoll-Hypaque gradient centrifugation. The mononuclear cells were washed three times in HBSS and resuspended for culturing.

Removal of marrow adherent cells. Adherent cells were removed by incubating 5×10^6/ml marrow mononuclear cells in HBSS plus 20% FCS in plastic Petri dishes (Nunc, Copenhagen, Denmark) for 3 h at 37°C in a fully humidified atmosphere of 7.5% CO_2 in air. Nonadherent cells, containing 2±1% ($n = 3$) monocytes, as determined by α-naphthylacetate esterase (ANA-esterase) staining, were collected, washed three times in HBSS, and resuspended for culturing. Marrow adherent cells consisted of 89±3% monocytes.

Colony assay. Marrow mononuclear cells, at a concentration of 1×10^5 cells/ml, were cultured in 1 ml modified Dulbecco's medium (16) supplemented with 10% FCS, 10 mg/ml BSA (Sigma Chemical Co., St. Louis, MO), 4×10^{-6} M human transferrin (Behring-Werke, Marburg, FRG) saturated with $FeCl_3$, 1×10^{-7} selenite (Merck, Darmstadt, FRG), 1×10^{-3} nucleosides (Sigma Chemical Co.), and 1.5×10^{-5} M each cholesterol and linoleic acid (Sigma Chemical Co.), using 0.3% agar (Difco Laboratories, Detroit, MI) as a semisolid agent. The level of IGF-I/Sm-C in culture medium containing all of the above constituents was 11 mU/ml. The level of hGH was < 0.1 ng/ml. 1-ml cultures were set up in 35- × 10-mm plastic Petri dishes (Falcon Plastics, Cockeysville, MD). As indicated, cultures were stimulated with Mo-conditioned medium (MoCM) as a source of GM colony-stimulating factor (GM-CSF) (17) or with 200 pM human recombinant GM-CSF (18), the latter kindly provided by Dr. Judith Gasson, UCLA School of Medicine, Los Angeles, CA. Various concentrations of IGF-I/Sm-C and hGH were added at the initiation of culture. Cultures were incubated at 37°C in a fully humidified atmosphere of 7.5% CO_2 in air. Colonies of > 50 cells were scored on the 14th d of culture.

Determination of colony types. An in situ staining technique in

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1. *Abbreviations used in this paper:* ANA-esterase, α-naphthylacetate esterase; ANOVA, one-way analysis of variance; CSF, colony-stimulating factor; GM-CFU, granulocyte/macrophage CFUs; GM-CSF, granulocyte/macrophage CSF; hGH, human growth hormone; IGF, insulin-like growth factor; IGF-I/Sm-C, IGF-I/somatomedin C; MoCM, Mo-conditioned medium.

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sequence for ANA-esterase (monocytes/macrophages) and naphthol-AS-D-chloroacetate esterase (granulocytes), performed as previously described (19), permitted typing of various colonies within the same culture dish.

Suspension cultures. Marrow mononuclear cells, at 0.5×10^6 cells/ml, were grown under virtually the same culture conditions as those used for the colony assay, except for the omission of agar. The cells were plated in 1 ml vol in 24-well dishes (Nunc) containing 200 pM recombinant GM-CSF and various concentrations of IGF-I/Sm-C or hGH. Cultures were incubated at 37°C in a fully humidified atmosphere of 7.5% CO_2 in air. After 7 d, the cells were cytocentrifuged, stained with Wright-Giemsa, and examined microscopically for 200-cell differential counts.

IGF-I/Sm-C and hGH. Biosynthetic (recombinant) human IGF-I/Sm-C (1 U/200 ng) and hGH were a gift from KabiVitrum, Stockholm, Sweden. The purity of IGF-I/Sm-C and hGH was 90 and 99%, respectively. Both peptide preparations were diluted in culture medium containing 0.1% BSA and added to the dishes at the initiation of culture.

Anti-IGF-I/Sm-C receptor monoclonal antibodies. An anti-IGF-I/Sm-C receptor monoclonal antibody, αIR-3 (20), was kindly provided by Prof. J. J. Van Wyk, University of North Carolina, Chapel Hill, NC. It was added at the initiation of culture at a final concentration of 15 μg/ml (100 nM). The addition of IGF-I/Sm-C and hGH to cultures containing αIR-3 was delayed for 2 h.

Statistics. Statistical analyses were performed using Student's *t* test or a one-way analysis of variance (ANOVA). Significance was defined as $P < 0.05$.

Results

Enhancement of colony formation by IGF-I/Sm-C and hGH. IGF-I/Sm-C enhanced the formation of colonies by human marrow GM-CFU (Fig. 1). Assayed in cultures stimulated with a saturating dose of MoCM, a significant increase in colony frequency was already detected at 6 ng/ml (30 mU/ml) IGF-I/Sm-C (131±8% of control GM-CFU frequency; $P < 0.001$). Maximal enhancement of colony formation (141±7% of control GM-CFU frequency) was observed at 60 ng/ml (300 mU) IGF-I/Sm-C (ANOVA, $P < 0.005$). Significant enhancement of human marrow GM-CFU by hGH (Fig. 2) was detected at a concentration of 150 ng/ml (129±14% of control GM-CFU; $P < 0.03$), being maximal (138±4% of control GM-CFU) at 250 ng/ml (ANOVA, $P < 0.005$).

Enhancement of human marrow myeloid colony formation by IGF-I/Sm-C and hGH was also detected in serum-free cultures stimulated with human recombinant GM-CSF (Table I). Although fewer colonies were detected under these culture conditions, elimination of serum did not significantly alter the concentration-dependent magnitude of colony enhancement induced by IGF-I/Sm-C and hGH. Myeloid colony formation was significantly enhanced in cultures stimulated with combined limiting concentrations of both IGF-I/Sm-C (2 ng/ml) and hGH (50 ng/ml) ($P < 0.05$), whereas combined maximal concentrations of both peptides did not exert an additive effect. The enhancement of myeloid colony formation in these cultures did not differ in magnitude from that observed in cultures stimulated with crude MoCM (Figs. 1 and 2).

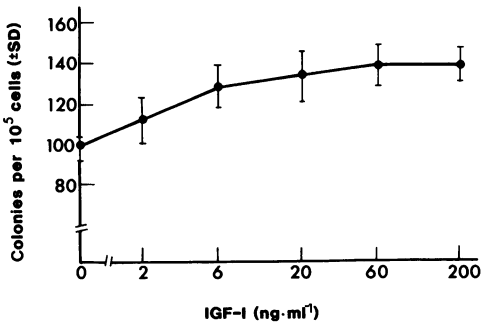

Figure 1. Effect of biosynthetic IGF-I/Sm-C on human marrow myeloid colony formation in vitro. Cultures were stimulated with 5% MoCM. No colonies grew in the absence of CSF. Results represent the mean±SD of four independent experiments, performed in triplicates.

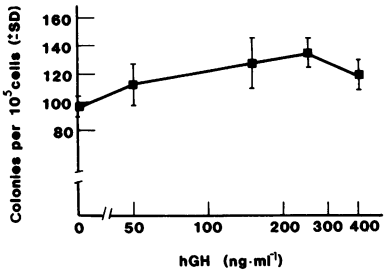

Figure 2. Effect of biosynthetic hGH on human marrow myeloid colony formation in vitro. Legends as in Fig. 1.

Interactions between IGF-I/Sm-C, hGH, and CSF. The mode of action of IGF-I/Sm-C and hGH in relation to CSF was evaluated by studying the effect of both peptides on colony formation by GM-CFU at various concentrations of MoCM, used as a source of CSF. As shown in a representative experiment (Fig. 3), IGF-I/Sm-C and hGH enhanced the growth of human marrow GM-CFU at both limiting and saturating concentrations of MoCM (Fig. 3 *A*), but did not alter the re-

Table I. Enhancement of Myeloid Colony Formation by IGF-I/Sm-C and hGH in Serum-free Cultures Stimulated with Human Recombinant GM-CSF

Additive	Colonies per 10^5 cells (±SD)	
	Serum free	Serum supplemented*
—	41±4 (100)‡	108±8 (100)
IGF-I/Sm-C (2 ng/ml)	45±5 (110±5)	120±5 (111±9)
IGF-I/Sm-C (6 ng/ml)	58±4 (138±8)	142±6 (131±6)
IGF-I/Sm-C (200 ng/ml)	62±5 (151±6)	149±9 (138±8)
hGH (50 ng/ml)	46±4 (112±5)	122±6 (113±4)
hGH (150 ng/ml)	53±6 (129±5)	136±9 (126±3)
hGH (250 ng/ml)	59±4 (144±6)	145±6 (134±6)
IGF-I/Sm-C (2 ng/ml) + hGH (50 ng/ml)	52±5 (122±4)	130±6 (120±5)
IGF-I/Sm-C (200 ng/ml) + hGH (250 ng/ml)	60±5 (146±6)	150±13 (139±7)

Cultures were stimulated with 200 pM human recombinant GM-CSF. The number of colonies in the absence of GM-CSF was 0 and 12±2 per 10^5 cells in the absence and presence of serum, respectively. Results represent the mean±SD of three independent experiments, performed in triplicates.

* Supplemented with 10% FCS.

‡ Numbers in parentheses indicate percent mean (±SD) colony frequency.

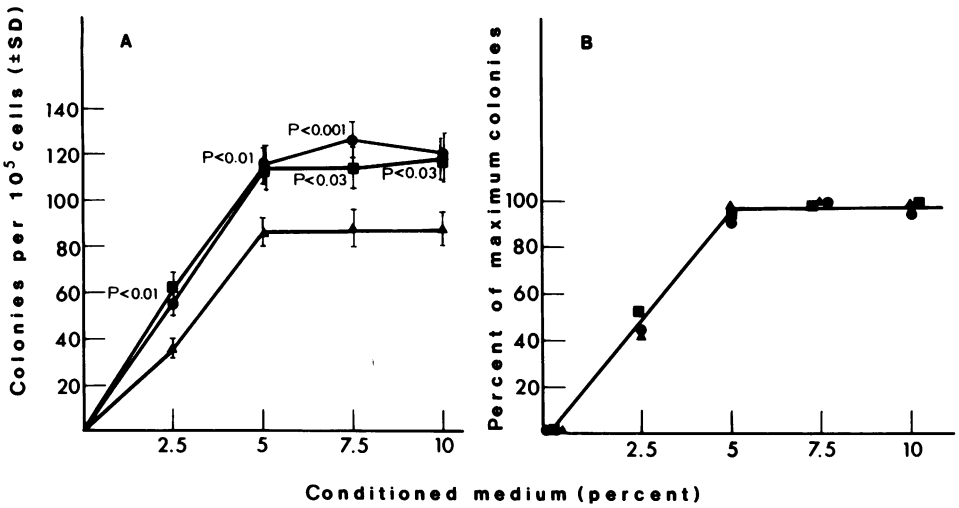

Figure 3. IGF-I/Sm-C and hGH in relation to CSF. (*A*) Absolute colony frequency. (*B*) Percentage of maximum colonies. Cultures stimulated with MoCM as a source of CSF (▲) were supplemented with either 200 ng/ml IGF-I/Sm-C (●) or with 250 ng/ml hGH (■). No colonies grew in the absence of CSF. Results represent the mean±SD of triplicate cultures.

sponsiveness of these progenitors to stimulation with CSF (Fig. 3 *B*). In addition, neither IGF-I/Sm-C nor hGH stimulated any colony formation when added in the absence of CSF to the cultures.

Colony types in cultures stimulated with IGF-I/Sm-C and hGH. Colony formation by GM-CFU results in the appearance of colonies that are composed of granulocytes, monocytes/macrophages, or both cell types (19). Using in situ staining techniques to determine colony composition, we examined the frequency of colony types that had developed in marrow cell cultures in the presence of various concentrations of IGF-I/Sm-C or hGH. As shown in Table II, both IGF-I/Sm-C (2–200 ng/ml) and hGH (50–250 ng/ml) induced a significant (~ 1.5–2.5-fold) increase in the number of colonies consisting only of granulocytes, whereas the total numbers of monocyte/macrophage or mixed granulocyte/macrophage colonies re-

Table II. Enhancement of Granulocyte Colony Formation by IGF-I/Sm-C and hGH

Additive	Serum	Colony types*		
		Granulocytes	Monocytes/ macrophages	Granulocytes/ macrophages
—	+	32±3	40±5	36±5
	−	12±2	17±2	12±3
IGF-I/Sm-C (2 ng/ml)	+	49±5 ($P < 0.05$)	37±2	34±2
	−	17±2	16±2	12±1
IGF-I/Sm-C (6 ng/ml)	+	70±3 ($P < 0.001$)	37±6	35±4
	−	29±3	16±3	13±2
IGF-I/Sm-C (200 ng/ml)	+	72±7 ($P < 0.001$)	40±3	37±6
	−	33±3	15±2	14±2
hGh (50 ng/ml)	+	43±5 ($P < 0.05$)	39±6	40±4
	−	18±3 ($P < 0.03$)	15±3	13±2
hGH (150 ng/ml)	+	56±5 ($P < 0.001$)	44±6	36±4
	−	22±4 ($P < 0.05$)	16±3	15±2
hGH (250 ng/ml)	+	67±7 ($P < 0.001$)	38±3	40±5
	−	29±4	15±2	15±2
IGF-I/Sm-C (2 ng/ml) + hGH (50 ng/ml)	+	53±7 ($P < 0.03$)	34±4	43±6
	−	23±4 ($P < 0.005$)	15±3	14±3
IGF-I/Sm-C (200 ng/ml) + hGH (250 ng/ml)	+	74±6 ($P < 0.001$)	35±3	41±7
	−	30±4	15±2	15±2

Data are given as colony frequency per 10^5 cells. Cultures were stimulated with 200 pM human recombinant GM-CSF. Results represent the mean±SD of three independent experiments, performed in triplicates. +, Supplemented with 10% FCS; −, serum free. * Colony types were calculated from the percentage of 100 to 200 typed colonies.

Table III. Effect of IGF-I/Sm-C and hGH on In Vitro Granulocytic Maturation

Additive	Serum	Percent cell type*			
		Promyelocytes	Myelocytes	Metamyelocytes	Band/segment
—	+	4±1	27±2	35±7	34±6
	−	0	25±3	37±6	38±4
IGF-I/Sm-C (2 ng/ml)	+	0	6±2 ($P < 0.001$)	39±9	55±7 ($P < 0.03$)
	−	0	9±2	36±8	55±6
IGF-I/Sm-C (6 ng/ml)	+	1±1	8±2 ($P < 0.001$)	40±8	51±6 ($P < 0.03$)
	−	2±1	14±3 ($P < 0.03$)	34±6	50±4
IGF-I/Sm-C (200 ng/ml)	+	2±1	9±2 ($P < 0.001$)	39±11	50±5 ($P < 0.03$)
	−	0	10±2	37±1	53±6
hGH (50 ng/ml)	+	2±1	12±2 ($P < 0.001$)	39±6	47±4 ($P < 0.05$)
	−	2±1	14±2 ($P < 0.03$)	32±4	52±4
hGH (150 ng/ml)	+	3±2	14±4 ($P < 0.001$)	37±6	46±4 ($P < 0.05$)
	−	0	8±2	41±9	51±6
hGH (250 ng/ml)	+	0	11±4 ($P < 0.001$)	40±9	49±6 ($P < 0.05$)
	−	1±1	13±3	33±7	53±4 ($P < 0.03$)

Data represent the mean±SD of three independent experiments, performed in duplicates. +, Supplemented with 10% FCS; −, serum free.
* Estimated by differential counts of 200 myeloid cells.

mained unchanged. The effect observed was virtually the same in both the presence and absence of serum.

Effect of IGF-I/Sm-C and hGH on granulocytic maturation in vitro. The effect of IGF-I/Sm-C and hGH on cellular maturation towards granulocytes was evaluated in 7-d suspension cultures of marrow mononuclear cells, in both the presence and the absence of serum. As compared with control cultures supplemented with GM-CSF alone (Table III), a significant decrease in the frequency of immature granulocytes (myelocytes) and an ~ 1.5-fold increase in the percentage of mature granulocytes (band/segment) was observed in the presence of IGF-I/Sm-C (2–200 ng/ml) and hGH (50–250 ng/ml). The frequency of metamyelocytes was not significantly altered. Differential counts of myeloid cells in control cultures supplemented with GM-CSF did not differ significantly from values of starting (day 0) marrow mononuclear cells (data not shown). The percentage of monocytes in all assay cultures did not differ from controls (14±2%). The viability in the harvested cell suspensions, determined by trypan blue exclusion, was > 85% in all cultures.

Role of adherent cells in enhancement of human marrow granulopoiesis. To evaluate whether the enhancement of granulopoiesis by IGF-I/Sm-C and hGH may be mediated by marrow accessory cells, the effect of both peptides was assayed in cultures that had been depleted of marrow adherent cells. Whereas IGF-I/Sm-C induced a greater than twofold augmentation in the total numbers of granulocyte colonies (Table IV), hGH failed to exert this effect in cultures of nonadherent, target marrow cells. In all cases, the total numbers of monocyte/macrophage and mixed granulocyte/macrophage colonies did not differ from control values (26±3 and 21±3 colonies/10^5 cells, respectively).

Whereas IGF-I/Sm-C induced a significant decrease in myelocytes and an increase in mature granulocytes in suspension cultures depleted of marrow adherent cells (Table V), this phenomenon was not detected in cultures supplemented with

Table IV. Effect of IGF-I/Sm-C and hGH on Myeloid Colony Formation by Nonadherent Target Marrow Cells

Additive	Colonies per 10^5 cells	
	Total frequency	Granulocyte colonies*
—	70±6	23±3
IGF-I/Sm-C (6 ng/ml)	90±4 ($P < 0.01$)	50±5 ($P < 0.001$)
IGF-I/Sm-C (200 ng/ml)	95±7 ($P < 0.01$)	53±6 ($P < 0.001$)
hGH (50 ng/ml)	69±6	22±2
hGH (250 ng/ml)	74±7	23±3

Cultures were stimulated with 200 pM human recombinant GM-CSF. No colonies were detected in the absence of GM-CSF. Results represent mean±SD of three independent experiments, performed in duplicates.
* Calculated from the percentage of 200 typed colonies.

Table V. Effect of IGF-I/Sm-C and hGH on Granulocytic Maturation in Suspension Cultures of Nonadherent Target Marrow Cells

Additive	Percent cell type*	
	Myelocytes	Band/segment
—	19±2	46±5
IGF-I/Sm-C (6 ng/ml)	13±3 ($P < 0.05$)	59±6 ($P < 0.05$)
IGF-I/Sm-C (200 ng/ml)	11±2 ($P < 0.01$)	62±6 ($P < 0.03$)
hGH (50 ng/ml)	24±4	42±4
hGH (250 ng/ml)	24±4	44±4

Results represent the mean±SD of three independent experiments, performed in duplicates.
* Estimated by differential counts of 200 myeloid cells.

Table VI. Abrogation of hGH-induced Enhancement of Granulocyte Colony Formation by an Anti-IGF-I/Sm-C Receptor Antibody, αIR-3

Additive	Colonies per 10^5 cells			
	Total colony frequency		Granulocyte colonies	
	−αIR-3	+αIR-3*	−αIR-3	+αIR-3
hGH (250 ng/ml)	146±8 ($P < 0.001$)	95±10	86±10 ($P < 0.001$)	37±6
IGF-I/Sm-C (200 ng/ml)	143±12 ($P < 0.001$)	108±13	82±12 ($P < 0.001$)	45±6
—	95±10	101±12	36±6	44±7

Cultures were stimulated with 200 pM human recombinant GM-CSF. hGH and IGF-I/Sm-C were added 2 h after the initiation of culture. Results represent the mean±SD of three independent experiments, performed in duplicates. * Added at a final concentration of 15 μg/ml.

hGH. The percentage of promyelocytes and myelocytes did not vary from control values (1±1 and 34±6%, respectively).

Role of IGF-I/Sm-C as mediator of hGH. The possibility that augmentation of marrow granulopoiesis by hGH may be mediated via IGF-I/Sm-C was investigated by the use of a monoclonal antibody (αIR-3) directed against IGF-I/Sm-C receptors.

αIR-3 abrogated the enhancement of granulocyte colony formation by hGH (Table VI) as well as its effect on granulocytic maturation in suspension cultures (Table VII). The enhancement of human marrow granulopoiesis by IGF-I/Sm-C was also eliminated by αIR-3, whereas colony growth and cellular maturation in control cultures remained unchanged. The presence of αIR-3 did not influence the total numbers of monocyte/macrophage and granulocyte/macrophage colonies in agar or the percentage of immature granulocytes (promyelocytes and myelocytes) in suspension cultures.

Discussion

Primarily regulated by specific CSFs (21), human hemopoiesis in vitro is also modulated by a variety of hormones and growth peptides (22–24). The growth-promoting effects of IGFs and hGH in human erythropoiesis have been extensively investigated and described (13–15, 25). The findings presented in this study demonstrate that IGF-I/Sm-C and hGH enhance the in vitro proliferation and maturation of human myeloid progenitor cells, as well.

IGF-I/Sm-C and hGH were found to enhance the formation of colonies in cultures of human marrow myeloid progenitors through a growth-promoting effect on granulopoiesis.

Table VII. Abrogation of hGH-induced Enhancement of Granulocyte Maturation by αIR-3

Additive	αIR-3*	Percent cell type	
		Myelocytes	Band/segment
hGh (250 ng/ml)	+	20±2	35±6
	−	12±2 ($P < 0.001$)	52±7 ($P < 0.03$)
IGF-I/Sm-C	+	18±3	39±5
(200 ng/ml)	−	11±3 ($P < 0.001$)	55±6 ($P < 0.03$)
—	+	22±2	34±4
	−	26±3	37±5

hGH and IGF-I/Sm-C were added 2 h after the initiation of culture. Results represent the mean±SD of three independent experiments, performed in duplicates. +, Present; −, absent.
* Added at a final concentration of 15 μg/ml.

Although our findings are at variance with a previous study claiming no effect of hGH on colony formation in cultures of human marrow GM-CFU (14), the phenomenon detected by us may have been masked by the presence of high concentrations (30%) of FCS, as serum is known to contain inhibitors of granulopoiesis (26, 27). A possible minor contribution of such inhibitors to our serum-supplemented (10% FCS) cultures is suggested by the similar effect of limiting doses of IGF-I/Sm-C on marrow granulocyte colony formation in both serum-free and serum-supplemented cultures, the latter containing detectable (11 mU/ml) IGF-I/Sm-C.

While serum-free cultures for studies of the regulation of hemopoiesis may exclude the interaction between a certain growth-promoting substance and any unknown variables (e.g., hormones, growth factors) that serum may contain, elucidation of the mechanism whereby such a substance exerts its effect upon hemopoietic progenitors is still complicated by the presence of accessory cells (e.g., fibroblasts, endothelial cells, monocytes, lymphocytes) within the bone marrow (28). Indeed, recent studies have shown that the enhancement of human marrow erythropoiesis in vitro by thyroid hormones is mediated indirectly by augmenting the release of soluble hemopoietic-like regulator molecules from accessory (probably B lymphocytes) marrow cells (29). Our findings have shown that IGF-I/Sm-C and hGH do not induce the production of specific hemopoietic regulators by bone marrow accessory cells (29–32), nor do they act as GM-CSF, since they do not stimulate the formation of colonies when added to culture in the absence of this regulator. However, the requirement of marrow adherent cells for enhancement of granulopoiesis by hGH, but not by IGF-I/Sm-C, revealed an indirect growth-promoting effect of the former on human marrow myeloid precursors. The possibility that marrow monocytes are the target cells that mediate the effect of hGH is suggested by their high frequency (89±3%) within the adherent cell population.

The functional link between IGF-I/Sm-C and hGH has been a subject of extensive investigation. IGF-I/Sm-C is considered to be the growth-promoting mediator of growth hormone (5), and although the liver was first singled out as a target for the stimulation of somatomedin production by growth hormone (33), it is now recognized that a variety of cells and tissues can respond to hGH by somatomedin synthesis, both in vivo (5, 34) and in vitro (35, 36). Our in vitro findings of abolishment of the growth-promoting effects of hGH on marrow myeloid progenitors by the anti-IGF-I/Sm-C receptor monoclonal antibody αIR-3 (20), provide strong evidence that IGF-I/Sm-C serves as the mediator of hGH among hemopoie-

tic progenitor cells as well. This is further supported by the strikingly similar effect of hGH and IGF-I/Sm-C on the growth and maturation of human marrow myeloid progenitors, as well as the combined effect observed at limiting and maximal concentrations of both peptides. That IR-3 did not alter in vitro granulopoiesis in control cultures indicates that the marrow cells themselves do not produce significant levels of IGF-I/Sm-C under basal conditions.

The effective concentrations of IGF-I/Sm-C for the phenomenon described in this investigation are within the physiologic range of normal serum concentrations (37) and do not differ in order of magnitude from those described for human and murine erythroid progenitors (13, 15). Maximal growth-promoting effects of hGH upon granulocyte colony formation were detected only at higher than physiologic concentrations. These findings may be explained by the use of culture conditions which, although appropriate for the growth of hemopoietic progenitors, may not be suitable for optimal functional expression of marrow accessory cells. Although the role of marrow adherent cells in hGH-induced enhancement of erythropoiesis in vitro has yet to be elucidated, the effective doses of hGH for granulocyte colony formation are in accordance with those recently described for erythroid progenitors by Claustres et al. (13).

IGF-I/Sm-C and hGH enhanced the maturation of morphologically recognizable granulocytic progenitors in suspension cultures of human marrow cells. We have recently found that these peptides also induced granulocytic maturation in suspension cultures of marrow cells from a patient with acute promyelocytic leukemia (manuscript in preparation). Although preliminary, these findings nevertheless warrant further extensive in vitro investigation as to the possible clinical application of IGF-I/Sm-C (or hGH) in the enhancement of myeloid progenitor cell maturation in neutropenic patients or in leukemia and preleukemia, alone or in concert with other maturation-inducing agents and drugs (38).

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