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Enhanced Response of Human Circulating Erythroid Progenitor Cells to hGH and to IGF-I in Children with Insufficient Growth Hormone Secretion

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ABSTRACT. The response of hematopoietic progenitors to the growth promoting effects of hGH and IGF-I has been documented. In this study, the effects of recombinant hGH and IGF-I on the growth of circulating erythroid burst forming cells (BFU-E) from growth-IGF-I retarded children with insufficient growth hormone secretion (IGHS) were evaluated and compared with values obtained from either children with short stature and normal growth hormone levels (SNGH) or normal donors. Both recombinant hGH and IGF-I had significantly greater stimulatory effects on the growth of BFU-E from the IGHS compared with the SNGH and with the normally growing children. At its optimal concentration of 200 μg/L, recombinant hGH had a stimulatory effect on the growth of BFU-E from 11 IGHS children yielding a mean ± SD value of 2.0 ± 0.3-fold above the unstimulated controls compared with 1.45 ± 0.16-fold and 1.36 ± 0.04-fold stimulation of BFU-E from six SNGH and five normal donors, respectively. Similarly, IGF-I, at its optimal concentration of 0.065 nmol/L (0.5 ng/mL), stimulated IGHS-derived BFU-E growth 1.67 ± 0.25-fold above unstimulated controls, compared with 1.28 ± 0.17-fold and 1.3 ± 0.1-fold stimulation of BFU-E from SNGH and from normal donors, respectively. The hGH- and IGF-I-induced stimulatory effects could be neutralized by their respective specific MAb. This significantly increased reactivity of erythroid progenitors from growth-retarded IGHS children to the erythropoietic effects of hGH and IGF-I may be the result of increased availability of cell surface receptors to these hematopoietic "synergistic" factors, secondary to their ambient decreased circulating concentrations *in vivo*. *In vitro* studies of peripheral blood BFU-E responsiveness to hGH and to IGF-I may be useful in implicating these peptides in growth retardation and possibly in predicting *in vivo* response to them. (*Pediatr Res* 32: 282–285, 1992)

Abbreviations

rEpo, recombinant erythropoietin
GH, growth hormone
BFU-E burst forming units-erythroid
IGHS, insufficient growth hormone secretion
SNGH, short stature with normal growth hormone secretion
PB, peripheral blood
rhGH, recombinant human growth hormone

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hGH and IGF-I are polypeptides that regulate proliferation and metabolic processes in a variety of mammalian cell types (1). Considerable evidence exists indicating an effect of these peptides in normal human erythropoiesis. Both hGH and IGF-I induce potentiation of colony formation by erythroid (BFU-E) as well as myeloid progenitor cells from normal human donors (2–5). IGF-I, which is presumed to mediate the effect of GH in various tissues, probably is the mediator of GH in erythropoiesis (4), although some reports suggest a direct effect of hGH on BFU-E (1, 2, 6). Studies on leukemic cell lines as well as on freshly obtained marrow cells from children with acute leukemia also indicate that hGH and IGF-I may enhance *in vitro* human leukemic cell proliferation (7).

Growth retardation in children often is associated with low circulating GH. No data as to the *in vitro* erythroid response to either hGH or IGF-I are available in those children. However, in growth-retarded children with Laron dwarfism, a disorder characterized by high levels of circulating GH, subnormal levels of circulating IGF-I, and unresponsiveness to hGH, peripheral BFU-E were found to be unresponsive to *in vitro* treatment with hGH (6). The present study was therefore undertaken to examine the response of erythroid progenitors from donors with insufficient GH secretion to the erythropoietic effects of hGH and IGF-I in comparison with the response of progenitors from donors with normal GH levels.

MATERIALS AND METHODS

Patients. Seventeen patients, eight boys and nine girls, between the ages of 6.5 and 14.5 y were studied. All patients had growth retardation with growth velocity below −2 SD for their chronologic age and gender. They were divided into two groups: Eleven (six boys and five girls) were diagnosed as having IGHS, based on their subnormal spontaneous 24-h GH serum integrated concentration levels (2.37 ± 0.71 μg/L) (8). Their GH responses to provocative tests with insulin, arginine, and clonidine (9, 10) were, however, within normal range (16.03 ± 9.2 μg/L). The second group of six children (two boys and four girls), whose GH serum integrated concentration and GH responses to provocative tests were normal (4.88 ± 1.48 and 29.73 ± 13.2 μg/L, respectively), were defined as children with short stature and normal GH, and together with five healthy, age-matched children of normal stature, served as a control group. Hb levels, reticulocyte counts, red blood cell counts, and mean corpuscular volume values were within normal range in both the study and the control groups. Participation of all children studied was contingent on obtaining parental informed consent, conforming with institutional standards and approved protocols for research involving human subjects.

Assay for BFU-E. Heparinized PB samples were layered on Ficoll-Hypaque gradients and centrifuged to obtain the mononuclear cell fraction. Circulating BFU-E were measured by a

modification of the assay of Dukes *et al.* (11). Mononuclear PB cells were plated at 5×10^5 in 1 ml of Iscove's modified Dulbecco's medium containing 30% FCS, 0.8% methylcellulose, and a stimulating dose of 1 unit of rEpo (Eprex, Cilag, Switzerland). The GH and IGF-I levels in FCS were <0.1 μg/L and <3 nmol/L, respectively. Cultures were incubated in a humidified atmosphere of 5% CO_2 at 37°C, and colonies were scored in duplicate cultures after 10 d. Before plating, cells were pulsed for 48 h at 37°C in 5% CO_2, in liquid medium with rhGH (Biotechnology, Rehovot, Israel) or IGF-I (Incstar, Stillwater, MN) at the indicated concentrations in the presence or absence of their respective antibodies [*i.e.* anti-hGH (Oris Industries, Yvepte, France) or anti-IGF-I (Incstar)]. Anti-hGH and IGF-I were added to the incubated cells at 1:200 and 1:1000 dilutions, respectively, 2 h before the addition of hGH or IGF-I.

Statistical analysis. Data are presented as the mean ± SD. The significance of difference between mean values was calculated using the *t* test.

RESULTS

Enhancement of BFU-E by rhGH, in rEpo-stimulated cultures, could be detected at a hormone concentration of 50 μg/L and was maximal at 200 μg/L (Fig. 1). It should be noted that BFU-E colony numbers in the control cultures (*i.e.* no hormone added) were 39 ± 12, 29 ± 7, and 30 ± 5 per $5 \cdot 10^5$ cells in the IGHS, SNGH, and normal donors groups, respectively, and were not significantly different ($p > 0.11$). Potentiation of BFU-E colony formation in cell cultures from the IGHS children, using 200 μg/L rhGH (2.0 ± 0.3-fold of control BFU-E; $p < 0.01$) was significantly greater ($p < 0.002$) than the increase observed in cell cultures from growth-retarded children with normal GH (1.45 ± 0.16-fold of control) and was also significantly greater ($p < 0.001$) than the increase observed in cultures from normal donors (1.36 ± 0.04-fold of control BFU-E). Furthermore, the frequency of the observed GH-induced increase in BFU-E growth of >1.5-fold was 91% (10 of 11) of the IGHS children, 33% (two of six) of the SNGH group, and none (zero of five) of the normal donors.

IGF-I-induced enhancement of BFU-E was detected at a concentration of 0.032 nmol/L (0.25 ng/mL) and was maximal at 0.065 nmol/L (0.5 ng/mL) (Fig. 2). Potentiation of BFU-E colony formation in cell cultures from the IGHS children, using 0.5 ng/mL recombinant IGF-I (1.67 ± 0.25-fold of control BFU-E; $p < 0.007$), was significantly greater ($p < 0.006$) than the increase observed in cell cultures from SNGH children (1.28 ± 0.17-fold of control) and was also significantly greater ($p < 0.009$) than the increase observed in cultures from normal donors (1.3 ± 0.1-fold of control) (Fig. 3). The frequency of the observed IGF-I-induced increase in BFU-E growth of >1.5-fold was 67% (six of nine) of the IGHS children, 17% (one of six) of the SNGH children, and none (zero of five) of the normal control group.

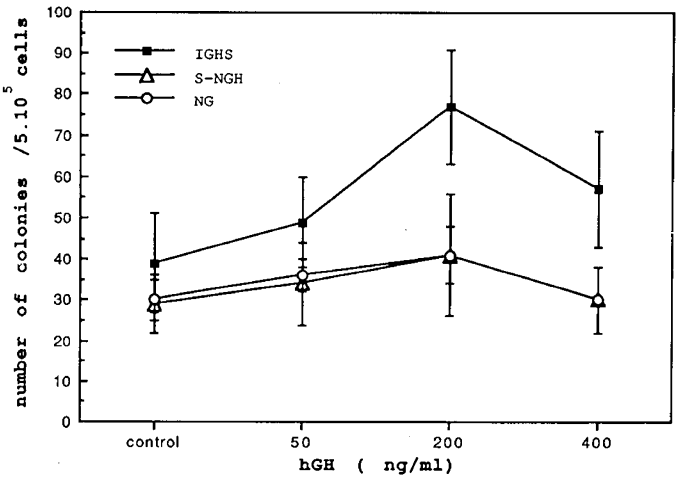

Fig. 1. Effect of hGH on development of BFU-E cultured *in vitro* from the peripheral blood of 11 patients with IGHS, six children with short stature and normal GH *S-NGH*, and five normal growing children *NG*. Cultures were stimulated with 1 unit of rEpo. Results represent the mean ± SD of all patients of each group, performed in duplicate. Conversion to SI units: hGH (μg/L) = (ng/mL).

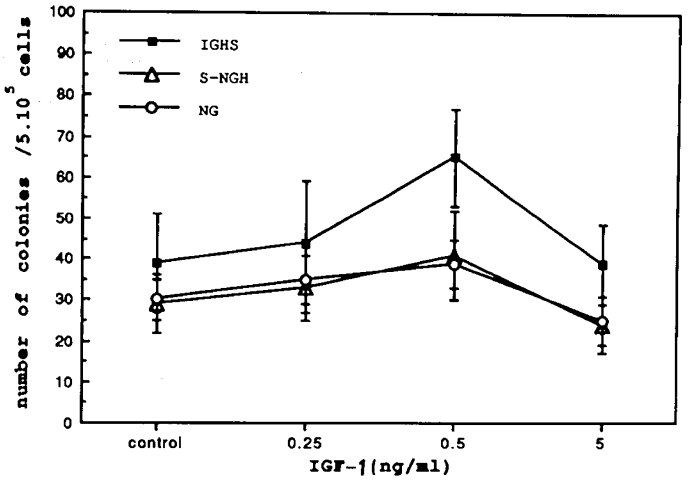

Fig. 2. Effect of IGF-I on development of BFU-E cultured *in vitro* from the PB of the studied patients. Conversion to SI units: IGF-I (nmol/L) = (ng/mL) × 0.1307. Abbreviations as in Figure 1.

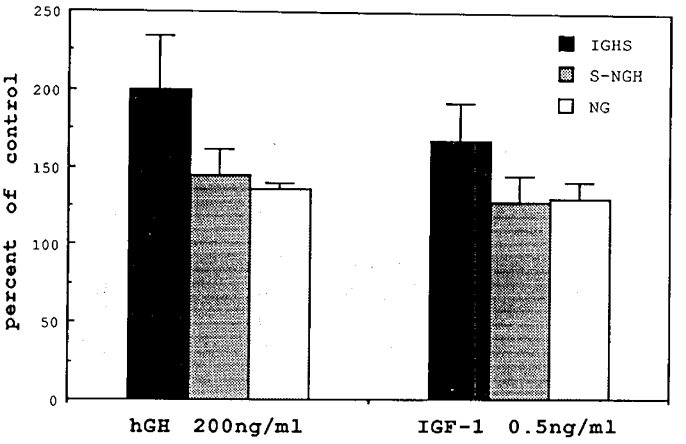

Fig. 3. Effect of hGH and IGF-I at their optimal concentrations on the growth of BFU-E from the PB of the studied patients, expressed as % of control (no hormone added). Abbreviations as in Figure 1.

The specificity of the described hormone-induced growth-potentiating effects was studied in the PB cultures of three IGHS patients and was demonstrated by the complete neutralization of the hGH-induced effect using an anti-hGH antibody (at a dilution of 1:200) and of the IGF-I-induced effect using an anti-IGF-I antibody (at a dilution of 1:1000) (Fig. 4).

DISCUSSION

Previous studies have demonstrated that hGH and IGF-I enhance *in vitro* proliferation of erythroid precursors (2, 4–6), and recent reports also have shown that both hGH and IGF-I enhance the *in vitro* growth of human bone marrow derived granulomonocytic colonies (3) and of leukemic blast colonies (7). Growth-retarded children with isolated GH deficiency are not regularly presented with significant anemia, although this finding has been postulated by at least one report (5). It has been long established, however, that hypophysectomized animals are anemic and that patients with pituitary malfunction suffer from a similar condition (12, 13). The anemia and the impaired DNA

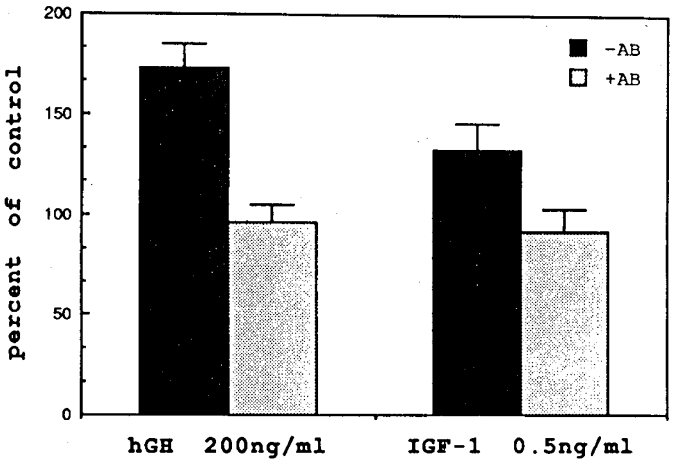

Fig. 4. Neutralization of hGH and IGF-I-induced enhancement of PB BFU-E growth by anti-hGH and IGF-I antibodies (*AB*). Each bar represents the mean ± SD of BFU-E from the peripheral blood of three IGHS patients expressed as % of control. AntihGH and IGF-I AB were added at 1:200 and 1:1000 dilutions, respectively.

and RNA synthesis in the bone marrow of hypophysectomized rats could be restored by GH (14), whereas treatment of prepubertal hypopituitary dwarfs with hGH in doses sufficient to induce linear growth induced increased hematopoiesis (13). In contrast, hGH failed to enhance erythropoiesis in acromegalic patients (15).

This study compares the response to hGH and to IGF-I of erythropoietic progenitor cells from 11 patients with insufficient GH secretion [a short-stature disorder characterized by a subnormal spontaneous GH secretion but a normal response to provocative tests, previously termed "neurosecretory dysfunction" (16)] with the response of cells from six growth-retarded patients with normal GH levels and five normally growing children. Focusing on growth-retarded children with IGHS as the study group seems to be appropriate because several reports have claimed that children with IGHS may represent a subset of growth-retarded patients with subnormal GH levels who could benefit from hGH therapy (8, 16, 17). Indeed, treatment of IGHS children for 2 y with hGH elicits a marked growth response similar to that noted in patients with a subnormal GH response to provocative tests (18). BFU-E from the IGHS children were significantly more responsive to the erythropoietic potentiating effects of both hGH and IGF-I than to BFU-E from the growth-retarded patients with normal GH levels or from normal donors.

The mechanism whereby hGH, alone or mediated by IGF-I, enhances the *in vitro* growth of human erythroid progenitors is not clear. Recent studies have shown that murine Friend erythroleukemia cells were specifically stimulated by GH (19) and, furthermore, that hGH acted as a potent direct stimulator of the human erythroleukemia cell line K562 (20). Indeed, membrane receptors for IGF-I have been detected in human erythrocytes (21).

Recently, attention has been directed toward several growth factors that do not stimulate hematopoietic colony formation directly but enhance the response of hematopoietic stem cells to specific proliferative and differentiative signals of terminal growth factors. GH and IGF-I may be classified as an example of those "synergistic hematopoietic growth factors" (22). It has been suggested that exposure to these synergistic hormones may be required for the initiation of DNA synthesis and hematopoietic cell division. Although the mechanism of this synergism is not clear, an attractive hypothesis, supported by some experimental evidence (23), suggests the induction of enhanced receptor expression in primitive stem cells, more of which then become responsive to specific inducers of terminal differentiation. The cause for the significantly greater response of erythroid progenitors from patients with insufficient GH secretion to the synergistic hematopoietic effect of both GH and IGF-I remains unresolved. Because clinically a correlation exists between the initial GH levels and the achieved linear growth in growth-retarded patients under hGH therapy (18), by analogy the increase in responsiveness of their hematopoietic cells to GH and to IGF-I may be the result of an increased availability of cell surface receptors to these peptides, secondary to their decreased circulating concentrations *in vivo*. In disorders associated with increased circulating GH levels such as acromegaly, no erythropoietic effect of GH could be detected (15). The performance of receptor-binding studies would have undoubtedly elucidated the mechanisms involved in the effects of "synergistic" factors in the enhancement of *in vitro* erythropoiesis in IGHS patients. Unfortunately, these were not performed in the present study because it is extremely difficult to isolate human PB hematopoietic progenitors in sufficient quantity for such studies. Changes in the BFU-E response to hGH and IGF-I in these patients, after hGH therapy, is under investigation.

These findings indicate that *in vitro* studies of erythroid progenitor responsiveness to GH and to IGF-I may be useful in implicating those peptides in growth retardation, as well as in predicting *in vivo* responses to them. In addition, the findings suggest a possible hematopoietic synergistic effect of GH or IGF-I in children with other disorders such as end-stage renal disease, a condition characterized by erythropoietin responsive anemia (24), in which growth retardation currently is being treated by hGH (25). The potential synergism between the hormones hGH and IGF-I and the hematopoietic growth factor rEpo on erythropoietic cell growth in these children is under investigation.

REFERENCES

1. Golde DW, Herschman HR, Lusis AJ 1980 Growth factors. Ann Intern Med 92:650–654
2. Golde DW, Bersch N, Li CH 1977 Growth hormone: species-specific stimulation of erythropoiesis *in vitro*. Science 196:1112–1113
3. Merchav S, Tatarsky I, Hochberg Z 1988 Enhancement of human granulopoiesis *in vitro* by biosynthetic insulin-like growth factor 1/somatomedin C and human growth hormone. J Clin Invest 81:791–797
4. Merchav S, Tatarsky I, Hochberg Z 1988 Enhancement of erythropoiesis *in vitro* by human growth hormone is mediated by insulin-like growth factor 1. Br J Hematol 70:267–271
5. Claustres M, Chatelain P, Sultan C 1987 Insulin-like growth factor 1 stimulates human erythroid colony formation *in vitro*. J Clin Endocrinol Metab 65:78–82
6. Golde DW, Bersch N, Kaplan SA, Rimoin DL, Li CH 1980 Peripheral unresponsiveness to human growth hormone in Laron dwarfism. N Engl J Med 303:1156–1159
7. Estrov Z, Meir R, Barak Y, Zaizov R, Zadik Z 1991 Human growth hormone and insulin-like growth factor-1 enhance lymphoblastic and myelogenous leukemia cell proliferation *in vitro*. J Clin Oncol 9:303–306
8. Zadik Z, Chalew SA, Kowarski AA 1985 Do short children secrete insufficient growth hormone? Pediatrics 76:355–360
9. Raiti S, Davis WT, Blizzard RM 1967 A comparison of the effects of insulin hypoglycemia and arginine infusion on release of human growth hormone. Lancet 2:1182–1183
10. Lanes R, Hurtado E 1982 Oral clonidine: an effective growth hormone releasing agents in prepubertal subjects. J Pediatr 100:710–714
11. Dukes PP, Ma A, Clemons GK, Meitas D 1985 Measurement of erythroid burst promoting activity by a specific cell culture assay. Exp Hematol 13:59–60
12. Crafts RC, Meineke HA 1959 The anemia of hypophysectomized animals. Ann NY Acad Sci 77:501–517
13. Jepson JH, McGarry EE 1972 Hemopoiesis in pituitary dwarfs treated with human growth hormone and testosterone. Blood 39:238–248
14. Nagy E, Berczi I 1989 Pituitary dependence of bone marrow function. Br J Haematol 71:457–462
15. Jepson J 1968 Agents influence the production and action of erythropoietin in man. J R Coll Phys 2:238–245
16. Spiliotis BE, August GP, Hung W, Sonis W, Mendelson W, Bercu BB 1984 Growth hormone neurosecretory dysfunction: a treatable cause for short stature. JAMA 251:2223–2230
17. Chalew SA, Raiti S, Armour KM, Kowarski AA 1977 Therapy in short children with subnormal integrated concentrations of growth hormone. Am J Dis Child 141:1195–1198
18. Zadik Z, Landau H, Rosenberg M, Limoni Y, Lieberman E 1992 Who will benefit from growth hormone therapy? Growth hormone therapy in short children. J Pediatr Endocrinol (in press)

19. Golde DW, Bersch N, Li CH 1978 Growth hormone modulation of murine erythroleukemia cell growth *in vitro*. Proc Natl Acad Sci USA 75:3437–3439
20. Gauwerky C, Golde DW, Li CH 1980 Growth hormone polypeptides stimulate proliferation of K562 human erythroleukemia cells. J Clin Endocrinol Metab 51:1208–1210
21. Polychronakos C, Guyda HJ, Posner BI 1983 Receptors for the insulin like growth factors on human erythrocytes. J Clin Endocrinol Metab 57:436–440
22. Quesenberry PJ 1986 Synergistic hematopoietic growth factors. Int J Cell Cloning 4:3–15
23. Neckers LM, Cossman J 1983 Transferrin receptor induction in mitogen stimulated human T lymphocytes is required for DNA synthesis and cell division, and is regulated by interleukin 2. Proc Natl Acad Sci USA 80:3493–3498
24. Sinai-Traiman L, Salusky IB, Fine R 1989 Use of subcutaneous recombinant human erythropoietin in children undergoing continuous cycling peritoneal dialysis. J Pediatr 114:550–554
25. Lippe B, Fine R, Koch VH, Sherman BM 1988 Accelerated growth following treatment of children with chronic renal failure with recombinant human growth hormone (somatrem): a preliminary report. Acta Paediatr Scand [Suppl] 343:127–131

Erratum

In the article "Oxygen-Related Prostaglandin Synthesis in Ductus Arteriosus and Other Vascular Cells" by Marlene Rabinovitch *et al.* (Pediatr Res 26:330–335, 1989), the labels on the left axes of Figure 3 were transposed. The corrected figure is shown below. We regret this error.