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The influence of growth hormone substitution therapy on erythroid and myeloid progenitor cells and on peripheral blood cells in adult patients with growth hormone deficiency

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Abstract. It has been reported that hypophysectomized rats exhibit normochromic, normocytic anaemia. Pancytopenia with impaired DNA synthesis in the bone marrow can be restored in these hypophysectomized rats by syngeneic pituitary grafts placed under the kidney capsule or treatment with growth hormone (GH). Until now, adults with hypopituitarism have received adequate replacement therapy with thyroxine, cortisol and sex steroids, but not with GH. We therefore investigated the effects of GH replacement therapy on the proliferation and differentiation of erythroid and myeloid progenitor and peripheral blood cells in 11 adult patients with growth hormone deficiency in a double-blind, placebo-controlled study for the first 6 months of therapy. The placebo group showed no changes during the first 6 months without therapy in either insulin-like growth factor I (IGF-I) levels, erythroid and myeloid progenitor precursor cells or peripheral blood cells. After commencement of GH therapy, IGF-I levels rose significantly during 24 months of therapy from $75{\cdot}3 \pm 13{\cdot}5$ to $225 \pm 34{\cdot}7\ \mathrm{ng\,mL^{-1}}$ ($P < 0{\cdot}001$). Erythroid and myeloid progenitor precursor cells showed a steep and significant increase after 18 and 24 months of therapy (erythroid: from $10{\cdot}7 \pm 3{\cdot}5$ to $261{\cdot}4 \pm 79{\cdot}8$, $P < 0{\cdot}02$, after 18 months and to $276{\cdot}8 \pm 149{\cdot}8 \times 10^5$ mononuclear cell colonies, $P < 0{\cdot}03$, after 24 months; granulocyte–macrophage colony-forming units: from $39{\cdot}7 \pm 9{\cdot}8$ to $316{\cdot}9 \pm 124{\cdot}6$, $P < 0{\cdot}002$, after 18 months and to $366 \pm 188{\cdot}7 \times 10^5$ mononuclear cell colonies, $P < 0{\cdot}03$, after 24 months), whereas the peripheral red and white blood cells exhibited only minimal non-significant changes. The principal regulators of erythropoiesis, such as erythropoietin, and parameters reflecting erythropoiesis in the peripheral blood, such as reticulocytes, remained almost unchanged throughout the whole study period. We therefore conclude that patients with GH deficiency do not have anaemia, but have haematopoietic precursor cells in the lower normal range, and that GH substitution therapy over a period of 24 months has a marked effect on erythroid and myeloid progenitor precursor cells but only negligible effects on peripheral blood cells in GH-deficient adults.

Keywords. Growth hormone deficiency, growth hormone substitution therapy, haematopoietic progenitor cells, peripheral blood cells.

Introduction

In children, GH deficiency results in retarded growth, and therapy with hGH has been established for many years. The consequences of GH deficiency in adults are less well known and have been described only recently. GH-deficient adults have decreased lean body mass, decreased life expectancy and an impaired emotional state [1–3]. Besides its effects on protein, carbohydrate and lipid metabolism, GH is known to influence haematopoiesis. Furthermore, GH and insulin-like growth factor I (IGF-I), in addition to their effects on hormones such as androgens, glucocorticoids, thyroxine, insulin and growth polypeptides [4–9], have been reported to potentiate the growth of erythroid precursor cells formed by bone marrow or peripheral blood erythroid cells in the presence of erythropoietin (Epo) [10–13]. Like monocytes, lymphocytes and several other tissues, erythrocytes have receptors for IGF-I and IGF-II [14], and it is thus hypothesized that the effect of hGH is mediated mainly by IGF-I. IGF-I can act via two possible routes: directly; and indirectly through enhanced erythropoietin production. It has been speculated that, as a consequence of a relative renal deficiency of oxygen resulting from an increase in kidney mass, enhanced Epo production will occur [12]. Opinions vary as to whether GH or IGF-I can influence human myeloid progenitor cells. Golde *et al.* [10] could find no effect of hGH on granulocyte/macrophage colony-forming units (CFU-C) or granulopoiesis even in concentrations up to $500\ \mathrm{ng\,L^{-1}}$ and showed that the effect of hGH on haemopoiesis of erythroid precursor cells is species specific. In contrast, Merchav *et al.* [15,16] showed that myeloid maturation was enhanced by adding hGH in the presence of marrow-adherent cells and mediated by a paracrine effect of IGF-I.

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The aim of this study was to determine whether GH replacement therapy would increase erythroid and myeloid progenitor cells and influence peripheral blood cells in adult patients with GH deficiency.

Materials and methods

Patients

Nineteen patients (15 women, four men) with a mean age of 45 ± 2·6 (range 27–60) years, all suffering from GH deficiency, entered this study after informed consent was given. The duration of GH deficiency had to be at least 2 years and was documented by an arginine-stimulated GH peak of less than 5 $\mu g\,L^{-1}$ after 2 h. In addition to their GH deficiency, nearly all of the patients had other pituitary deficiencies and received adequate replacement therapy, which remained unchanged throughout the study.

Patient characteristics are given in Table 1. Patients were examined and blood was drawn at 0, 3, 6, 9, 12, 18 and 24 months after the start of the study.

Study design

The study was randomized, double blind and placebo controlled (GH/placebo) for the first 6 months. Thereafter, all patients received GH (Genotropin, Pharmacia, Stockholm, Sweden), and patients in the placebo group were added to the treatment group. Only 11 of the original 19 patients were observed over 24 months; the other patients dropped out. Patients from the placebo group ($n = 4$) were tested over a period of 30 months from the start of the study, i.e. over a period of 24 months of GH therapy. Placebo was supplied in identical cartridges for reconstitution with 1 mL of water for injection with 3 mg of m-cresol.

Treatment schedule

The rhGH dose was 0·125 IU kg^{-1} per week during the first 4 weeks and thereafter 0·25 IU kg^{-1} per week. In two of the remaining 11 patients, the dose had to be reduced to 0·125 IU kg^{-1} per week, and in one patient to 0·08 IU kg^{-1} per week. The weekly dose was divided into seven daily s.c. injections. Irrespective of body weight the maximum dose per day did not exceed 4 IU.

Adverse events

Three patients suffered from myalgias (two in the therapy group and one in the placebo group), six patients suffered from arthralgias (four in the therapy group and two in the placebo group), seven patients showed peripheral oedema (five in the therapy group and two in the placebo group) and one patient in the placebo group suffered from newly developed headache. Eight patients (seven in the therapy group and one in the placebo group) stopped the therapy during the 2-year treatment period. A reduction in the drug dose was necessary in three out of the remaining 11 patients.

IGF-I was measured with a radioimmunoassay method after serum treatment with acid ethanol to precipitate and neutralize the IGF-I-binding proteins in accordance with Blum *et al.* [17]. The minimum detectable IGF-I concentration was 20 ng mL^{-1} and the intra- and interassay coefficients of variation were 3·1% and 10% respectively.

Preparation of cells

Peripheral blood (PB; 5 mL) for the evaluation of haematopoietic progenitor cells was collected into 20-mL sterile tubes containing 1 mL of EDTA. From this, PB mononuclear cells (MNCs) were extracted and harvested after a Ficoll–Hypaque gradient centrifugation (400 × *g* for 40 min, 1·077 g mL^{-1}).

Progenitor cell assays in the peripheral blood

Granulocyte–macrophage colony-forming units, erythroid burst-forming units (BFUe) and CFU-mix progenitor cells were assayed using a modification of the clonal assay described by Fauser & Messner [18]. Each plate contained 0·9% methylcellulose, 30% fetal calf serum (FCS), 10% bovine serum albumin (BSA; Behring, Marburg, Germany), α-thioglycerol (10^{-4} mol L^{-1}), 5% phytohaemagglutinin–leucocyte-conditioned medium (PHA–LCM) and Iscove's modified Dulbecco medium (IMDM; Gibco, Paisley, UK). For cultivation of BFUe and CFU-mix, 1 U mL^{-1} erythropoietin (Epo; Toyoba, Osaka, Japan) was added to culture dishes. PBMNCs were plated in triplicate at 0·7–2·0 × 105 mL^{-1}. After a culture period of 14 days (37°C, 5% CO_2, full humidity), cultures were examined under an inverted microscope. Aggregates of more than 40 translucent, dispersed cells were counted as CFU-GM. Bursts containing more than 100 red-coloured cells were scored as BFUe. CFU-mix were identified by their heterogeneous composition of translucent and haemoglobinized cells. All colonies suspected as being CFU-mix were picked, transferred to glass slides and stained with May–Grünwald Giemsa for cytological examination under a light microscope.

The total number of MNCs per millilitre of PB was determined by multiplying the leucocyte count by the percentage of MNCs in the differential count. The MNC fraction consists of monocytes, lymphocytes and immature myeloid forms circulating in the PB. The total numbers of haematopoietic progenitors per millilitre of PB were then determined by multiplying the number of colonies per 10^5 MNCs by the total number of MNCs per millilitre of PB [19].

Haematological examinations

For haematological examinations, blood was collected in EDTA-coated tubes. Parameters measured included the total counts of red blood cells (RBCs), white blood cells (WBCs) and platelets, and determination of haemoglobin and haematocrit (Sysmex 2000; TOA, Tokyo, Japan). Differential blood cell counts were established on

Table 1. Patients

ID	Sex	Age (years)	Diagnosis	Other pituitary dysfunction	Hormone replacement therapy
LH*	F	48	Endocrine-inactive pituitary adenoma	Gonadotropic, thyreotropic, corticotropic	Oest/gest, L-thyroxine (100 μg), hydrocortisone (10 mg)
TC*	F	55	Endocrine-inactive pituitary adenoma	Gonadotropic, thyreotropic, corticotropic	L-thyroxine (100 μg), hydrocortisone (20 mg)
HC*	F	56	Prolactinoma	Gonadotropic, thyreotropic, corticotropic	L-thyroxine (100 μg), hydrocortisone (20 mg)
SC*	F	45	Prolactinoma	Gonadotropic	Oestradiol/gestagen
BH*	M	31	Prolactinoma	Gonadotropic, thyreotropic, corticotropic	L-thyroxine (100 μg), prednisone (5 mg)
GP*	M	54	Prolactinoma	–	–
AG	F	39	Prolactinoma	Gonadotropic	–
HE*	F	52	Craniopharyngioma	Gonadotropic, thyreotropic	L-thyroxine (100 μg)
KM*	F	28	Idiopathic GH deficiency	Thyreotropic	L-thyroxine (100 μg)
CE*	F	43	Endocrine-inactive pituitary adenoma	Gonadotropic, thyreotropic, corticotropic	Oest/gest, L-thyroxine (100 μg), hydrocortisone (25 mg)
GU*	F	49	Prolactinoma	Gonadotropic	Oestradiol/gestagen

Oest/gest, oestradiol/gestagen.

the examination of 200 cells of a Giemsa-stained blood smear by two independent observers.

Erythropoietin assay

Erythropoietin levels were measured by a commercially available monoclonal enzyme-linked immunosorbent assay (ELISA), standardized against the second international preparation of Epo for bioassays (Medac, Hamburg, Germany). The serum level in healthy subjects ranges from 5 to 25 mU mL^{-1}. In addition, results were correlated with a modified specific radioimmunoassay. The exact procedure has been published previously [20].

Statistical methods

Wilcoxon's signed-rank test, as implemented in the SAS Univariate procedure (SAS Institute, 1990), was used to test whether the changes in the parameters measured before and after therapy differed significantly from zero. All P-values are based on two-sided tests and the differences were considered significant at $P < 0{\cdot}05$.

Results

During the first 6 months, patients in the control group receiving placebo ($n = 9$) showed no changes in IGF-I levels ($88{\cdot}7 \pm 44$ vs. $81{\cdot}5 \pm 50$ ng mL^{-1}), red blood cells or haematopoietic precursor progenitor cells. During the same period, IGF-I levels in the treatment group ($n = 10$) rose significantly from $74{\cdot}8 \pm 13{\cdot}6$ to $192{\cdot}4 \pm 25{\cdot}5$ ng mL^{-1} ($P < 0{\cdot}001$) without changes in PB cells or haematopoietic precursor progenitor cells. After 6 months, all patients received GH therapy, and the patients were pooled into one group of 11 patients. IGF-I levels rose significantly from $75{\cdot}3 \pm 13{\cdot}5$ to $225 \pm 34{\cdot}7$ ng mL^{-1} ($P < 0{\cdot}001$, Fig. 1) after 24 months of therapy, whereas erythrocyte counts, haemoglobin and haematocrit showed only minimal insignificant changes during 24 months of therapy (Fig. 2). The peripheral white blood cells remained stable throughout the whole period of therapy (data not shown).

Regarding erythroid and myeloid progenitor cells, we observed a steep and significant increase in BFUe and

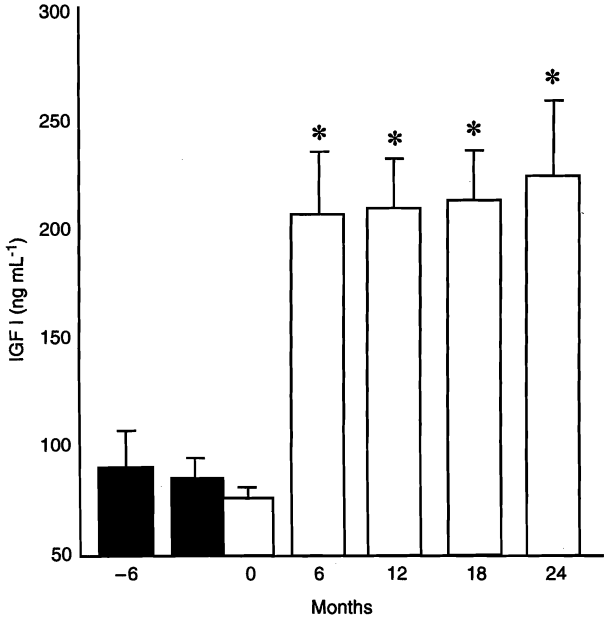

Figure 1. Time course of IGF-I levels in adult patients with GH deficiency. Nine patients received placebo for 6 months (shaded bars) and 11 patients received hGH over a period of 24 months (open bars). *$P < 0{\cdot}001$.

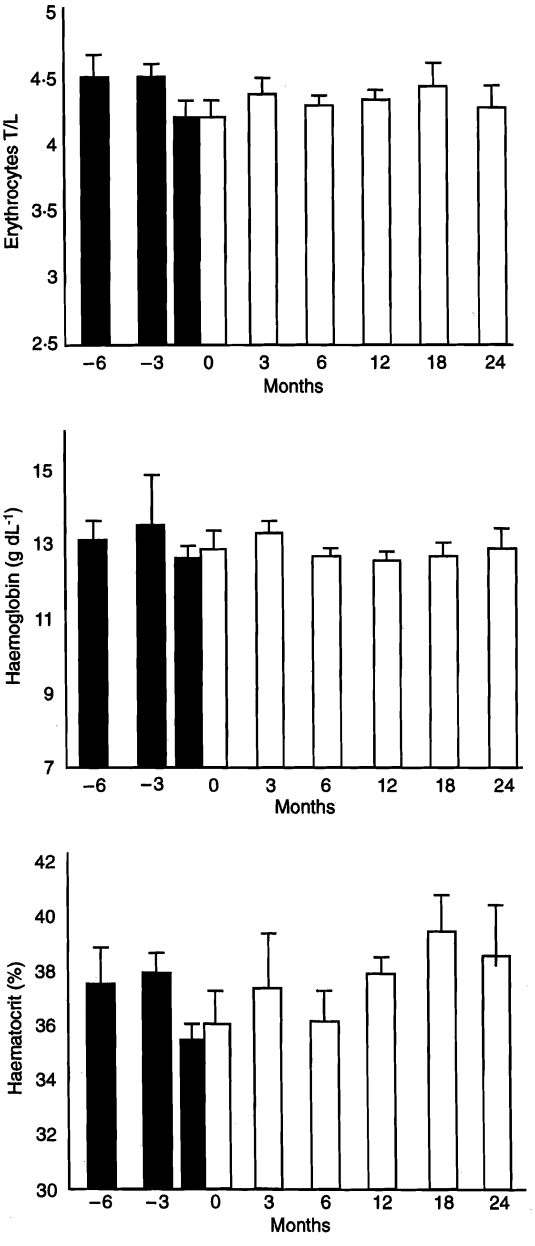

Figure 2. Time course of (A) erythrocytes, (B) haemoglobin and (C) haematocrit in adult patients with GH deficiency. Nine patients received placebo for 6 months (shaded bars) and 11 patients received human growth hormone (hGH) over a period of 24 months (open bars).

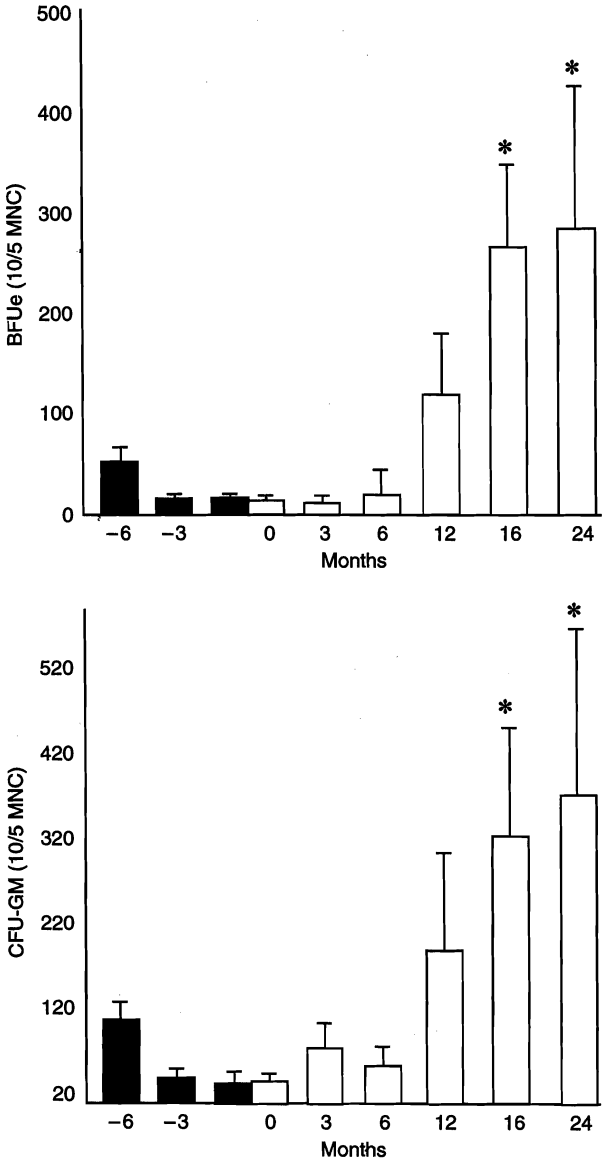

Figure 3. Time course of (A) burst-forming units erythroid (BFUe) and (B) granulocyte–macrophage colony-forming units (CFU-GM) in adult patients with GH deficiency. Nine patients received placebo for 6 months (shaded bars) and 11 patients received human growth hormone (hGH) over a period of 24 months (open bars). *$P < 0{\cdot}03$.

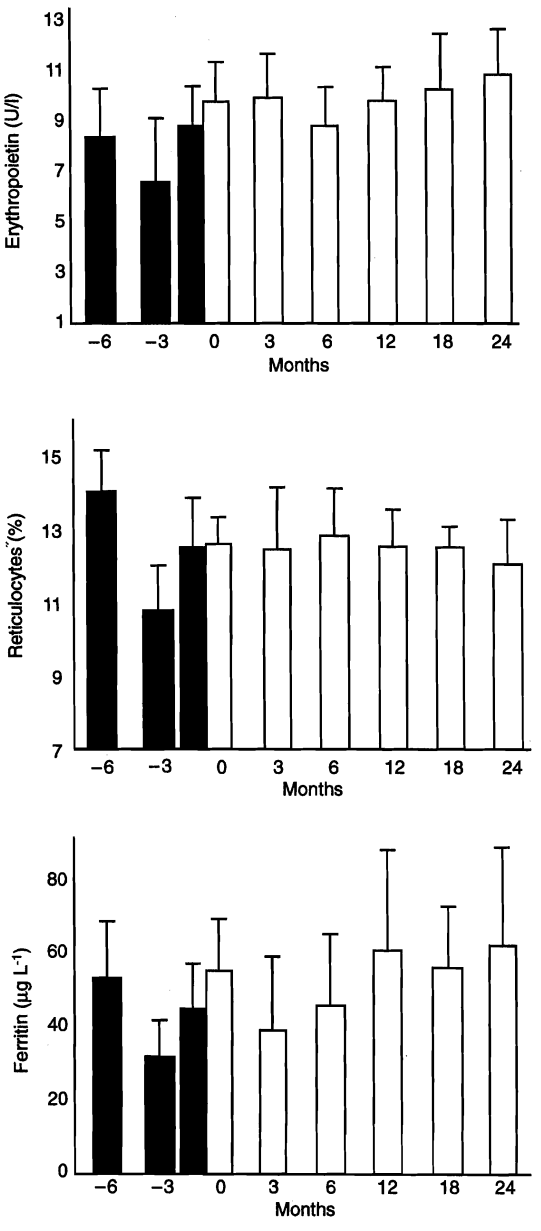

Figure 4. Time course of (A) erythropoietin, (B) reticulocytes and (C) ferritin in adult patients with GH deficiency. Nine patients received placebo for 6 months (shaded bars) and 11 patients received hGH over a period of 24 months (open bars).

CFU-GM after 18 months and 24 months of therapy. (BFUe rose from $10{\cdot}7 \pm 3{\cdot}5$ to $261{\cdot}4 \pm 79{\cdot}8$, $P < 0{\cdot}02$, after 18 months and to $276{\cdot}8 \pm 149{\cdot}8 \times 10^5$ colonies MNC, $P < 0{\cdot}03$, after 24 months; CFU-GM rose from $39{\cdot}7 \pm 9{\cdot}8$ to $316{\cdot}9 \pm 124{\cdot}6$, $P < 0{\cdot}002$, after 18 months and to $366 \pm 188{\cdot}7 \times 10^5$ colonies MNC, $P < 0{\cdot}03$, after 24 months), whereas only slight changes occurred during the first 12 months of therapy (Fig. 3).

No significant correlations between IGF-I and the haematopoietic precursor cells were found.

Erythropoietin, the main regulator of human erythropoiesis, and reticulocytes did not change significantly during the period of treatment in both groups. Ferritin also remained stable (Fig. 4).

Discussion

Treatment with GH has been shown to restore body composition, to reduce high waist-to-hip ratios, to increase muscle strength and exercise capacity, to lower fasting plasma cholesterol levels [1,21] and to increase bone mineral density in adult patients with acquired GH deficiency [22]. In addition, hypophysectomized animals with pituitary deficiency suffer from anaemia with an impaired DNA and RNA synthesis in the bone marrow, and pancytopenia can be restored by syngeneic pituitary grafts or GH therapy [23].

GH and IGF-I are also known to potentiate the stimulating effect on erythroid progenitor precursor

cells *in vitro* and *in vivo* in the presence of erythropoietin [10–13]. A clear effect on the bone marrow *in vitro* was detectable at GH concentrations of $5\,ng\,mL^{-1}$, with a maximum stimulation at $50–100\,ng\,mL^{-1}$. However, it appears that the growth-promoting effect of GH on late erythroid precursor cells is mediated through IGF-I, because receptors for IGF-I and IGF-II have been found on rat and human erythrocytes [14] and the effect can be blocked by a monoclonal anti-IGF-I receptor antibody [13,15]. Furthermore, to achieve an increase in erythroid precursor cells, supraphysiological concentrations of GH, but only physiological concentrations of IGF-I, are needed [11]. Other experiments also have shown that physiological concentrations of insulin have a synergistic effect with IGF-I on the enhancement of erythroid colony-forming units (CFUe), reducing the amount of IGF-I necessary for this effect [24]. Other authors have shown that expansion of the pool of erythroid precursor cells (mainly CFUe), unlike that of myeloid precursor cells, does not require accessory cells and the release of soluble haematopoietic-like regulator molecules in the bone marrow [25]. Erythroid precurors mainly need IGF-I or insulin as well as Epo for the production of erythroid cells, and their activities are mediated by a similar receptor or post-receptor system [26].

It has been reported that hypopituitary rats have low Epo levels and that IGF-I causes a significant rise in Epo levels [12]. Two possible routes of action of IGF-I have been postulated—a direct one and an indirect one via enhanced erythropoietin production.

So far, to the best of our knowledge, no clinical study has examined the effects of long-term GH substitution therapy in adult patients with GH deficiency on haematopoietic progenitor precursor and peripheral blood cells. In our study, GH therapy caused a significant increase in IGF-I levels after 6 months of treatment, whereas IGF-I remained stable in the placebo group and rose significantly only after GH therapy was started. We could demonstrate a significant and marked increase in erythroid and myeloid progenitor precursor cells with marginal, but not significant, changes in peripheral blood cells after 18 and 24 months of therapy. The late onset of the effect might be caused by the absence of anaemia and thus the lack of need for immediate action. Additionally, the marginal effect on peripheral blood cells might be due to an increase in plasma volume masking higher erythrocyte, haemoglobin and leucocyte counts. The placebo (control) group showed a slight decrease in haematopoietic progenitor and peripheral red blood cells in the first 6 months, whereas, despite significantly higher IGF-I levels, progenitor and peripheral blood cells remained stable in the therapy group during this time. Erythropoietin rose slightly during the 24-month treatment period, supporting the hypothesis that erythropoiesis in these patients is additionally affected by enhanced Epo production.

A threefold higher concentration of IGF-I has been reported to be necessary for a growth-promoting effect on CFUe compared with BFUe, suggesting that there is a gradual loss of sensitivity during the process of erythroid maturation [13]. This would be in accordance with the results of our study, in which we observed a marked effect of hGH on erythroid progenitor precursor cells after 18 and 24 months of therapy, but no effect on the number of peripheral red and white blood cells.

As none of our patients showed anaemia despite long-term GH deficiency, we speculate that GH produced locally by PMNCs [27], which has the same biological effect as pituitary GH, might compensate for the low pituitary GH concentrations in these patients. Thus, these patients are not completely GH deficient, and this low concentration may have sufficient biological effect to prevent anaemia. The present data also confirm the results of Merchav *et al.* [15,16] demonstrating that GH has a stimulatory effect on granulocyte progenitor cells. The unchanged number of peripheral granulocytes could be explained by the need for supraphysiological GH concentrations to exhibit changes in granulocytes. In our patients, the influence of other endocrinological factors could be excluded by stable adequate substitution therapy.

GH plays a regulating role in haematopoiesis not only in GH-deficient adults, but also in patients suffering from renal failure. In addition to several other factors influencing haematopoiesis, such as decreased Epo production, shortened red blood cell survival and the presence of toxic substances, GH is also considered to play a major role in regulating erythropoiesis in uraemic patients [28]. In this group of patients, IGF-I was found to be directly correlated with haematocrit. In healthy male and female rats, IGF-I was also positively correlated with an increase in red cell formation during accelerated growth, and it was shown that Epo affects the expansion of red cell volume in this period [29].

In conclusion, the present data suggest that patients with GH deficiency do not have anaemia, but have levels of haematopoietic precursor cells in the lower normal range. This long-term study in adult patients with acquired GH deficiency treated with GH for 24 months strengthens previous *in vitro* reports suggesting that GH and IGF-I have a stimulatory effect on erythroid and myeloid precursor progenitor cells but only a marginal effect on peripheral red and white blood cells. This marginal effect might be caused by an expansion of plasma volume as a result of GH therapy. It seems that GH is one of several hormones that can potentiate the cellular response of Epo on haemopoiesis.

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