CLINICAL STUDY

Serum cytokine levels in GH-deficient children during substitutive GH therapy

Sara Pagani, Cristina Meazza, Paola Travaglino, Fabrizio De Benedetti¹, Carmine Tinelli² and Mauro Bozzola

Dipartimento di Scienze Pediatriche, Università degli Studi di Pavia, Piazzale C Golgi 2, 27100 Pavia, Italy, ¹Direzione Scientifica, IRCCS Ospedale Pediatrico Bambino Gesù, Rome, Italy and ²Biometrics Unit, IRCCS San Matteo, Pavia, Italy

(Correspondence should be addressed to M Bozzola; Email: m.bozzola@smatteo.pv.it)

Abstract

Objective: The aim of the present study was to investigate the effect of exogenously administered human GH (hGH) on serum levels of interleukin (IL)-4, IL-6, IL-12 and tumour necrosis factor (TNF)- α in GH-deficient (GHD) children.

Design and methods: We evaluated 13 short prepubertal GHD children, aged between 2 and 13 years, and 13 age-matched healthy subjects as controls. Circulating cytokine values were evaluated in basal conditions in all children, and 6 and 24 h following the 1st hGH injection (0.23 mg/kg per week), and then after 3 months of hGH treatment in GHD patients. Serum levels of IL-4, IL-6, IL-12 and TNF- α were measured by commercially available ELISAs.

Results: No significant differences were found between controls and GHD children in basal values of serum IL-4, IL-6, IL-12 and TNF- α ($P > 0.05$ by Mann–Whitney U test). Analysis of cytokine levels during hGH treatment showed significant changes over time in TNF- α and IL-6 levels ($P = 0.0014$ and $P = 0.00024$ respectively), with the more pronounced effect observed at 6 h following the first administration of hGH (i.e. increase in IL-6 (Wilcoxon matched pairs test, $P = 0.0015$) and TNF- α levels ($P = 0.0015$)). No significant changes over time were observed in IL-4 and IL-12 serum levels.

Conclusions: *In vivo* release of the pro-inflammatory cytokines IL-6 and TNF- α can be affected by hGH treatment in GHD children, suggesting a direct effect of GH on the immune function.

European Journal of Endocrinology 152 207–210

Introduction

Growing evidence indicates a bidirectional relationship between the neuroendocrine system and immune function. Neurohormones act on immune cells while cytokines, secreted by lymphocytes and macrophages, in turn influence the neuroendocrine system. The variability of endocrine and immune responses depends on these interactions, which can be facilitatory or inhibitory. The presence of cell surface receptors for growth hormone (GH) and insulin-like growth factor-I (IGF-I) on different lymphocyte subpopulations suggests that, in addition to the endocrine effects, there is an immune action for these hormones (1, 2). Indeed, a relationship between GH and the immune system is suggested by *in vivo* studies, in particular in experimental animals. In humans, no obvious immune deficit has been found in GH-deficient (GHD) patients (3, 4). However, supporting the effect of GH on immune cells, the release of interleukin (IL)-1 β and tumour necrosis factor- α (TNF- α) is increased in response to an acute GH administration in GHD children (5). Furthermore,

cytokine release can be affected by short-term human GH (hGH) administration in short non-GHD children indicating a direct effect of GH on the immune function (6). The aim of the present study was to evaluate the effect of short- and long-term hGH administration on *in vivo* production of a large body of cytokines, by monitoring serum IL-4, IL-6, IL-12 and TNF- α concentrations in GHD children, before and during hGH treatment.

Patients and methods

Patients and study design

We evaluated 13 children (nine males and four females), aged between 2 and 13 years, with isolated complete ($n = 6$) and partial ($n = 7$) GH deficiency, documented by serum GH peak levels less than 5 ng/ml and 5–10 ng/ml respectively, in response to at least two pharmacological stimuli. A group of 13 children (six males and seven females), aged between 3 and 11 years, were studied as controls; they were taking part in a screening

programme for hyperlipidaemia performed at school. In patients, the pituitary–thyroid axis function, evaluated by serum tri-iodothyronine, thyroxine, free tri-iodothyronine, free thyroxine and thyrotrophin levels, was normal; the adrenal function, estimated on the basis of basal serum cortisol values at 0800 and 2000 h, was also normal. No child suffered from diabetes insipidus, chronic diseases or acquired GH deficiency or had chromosomal abnormalities. Informed consent was obtained from the parents of all the children. Serum samples for cytokine measurement were obtained at 0900 h both in GHD children and in age-matched controls. In addition, in GHD children they were also obtained 6 and 24 h following the 1st hGH injection (0.23 mg/kg per week), and then after 3 months of GH treatment. GH was self-administered by the patients as a daily injection in the evening at bedtime. Blood samples were placed on ice immediately and centrifuged within 1 h. Sera were stored at -20°C until tested for cytokine content.

Measurement of cytokine levels

Serum levels of IL-4, IL-6, IL-12 (p70) and TNF- α were measured by commercially available ELISAs (ELISA DuoSet; R&D Systems, Minneapolis MN, USA). The minimum detectable concentrations by these assays were 7 pg/ml for TNF- α , 2.34 pg/ml for IL-6, 15 pg/ml for IL-4 and 15 pg/ml for IL-12. The samples were diluted 1/5 for TNF- α , 1/10 for IL-6, 1/3 for IL-4 and 1/5 for IL-12 in assay buffer. The intra- and inter-assay coefficients of variation (C.V.) were less than 7%.

Measurement of IGF-I levels

Serum IGF-I concentrations were measured by double-antibody RIA using immunochemicals and tracer provided by Medgenix (Fleurus, Belgium). The sensitivity of the assay was 150 pg/ml; the intra- and inter-assay C.V. ranges were 3.9–6% and 5.6–7.5% for a quality control range of 30–450 $\mu\text{g/l}$ respectively. In order to avoid interference from binding proteins, single plasma EDTA samples were treated with acid ethanol.

Statistical analysis

Data are presented as medians (and interquartile range) and non-parametric tests were employed in the statistical analysis of the data because of the small sample size and because the data were not normally distributed (Shapiro–Wilk's test). The Mann–Whitney U test was used to compare unpaired data between two groups, while Friedman (in cases of more than two times), ANOVA and Wilcoxon (in cases of comparison of the same group twice) tests were used to compare paired data. Correlations were analyzed using the Spearman rank correlation test. All tests were two-sided. A P value <0.05 was deemed statistically significant. The analyses was performed with Statistica

6.0 for Windows software (StatSoft, Inc. 2003, Tulsa, OK, USA).

Results

In order to evaluate the influence of hGH therapy on serum cytokine levels in GHD children, we measured IL-4, IL-6, IL-12 and TNF- α concentrations in blood samples collected before and after 6 h, 24 h and 3 months of treatment in patients. Basal values were compared with those of healthy children. No significant differences in serum levels of IL-4, IL-6, IL-12 and TNF- α before hGH treatment were observed between GHD children and controls comparable for age (Table 1). As expected, treatment with GH resulted in a significant increase over time in plasma IGF-I levels (Friedman ANOVA test χ^2 ($n = 13$, degrees of freedom = 3) = 20.74; $P = 0.00012$); with all individual patients showing a marked increase in circulating IGF-I levels and velocity rate of growth 1 year after the beginning of the treatment (data not shown). This showed that in all patients GH treatment was associated with a biological effect. Analysis of cytokine levels during GH treatment showed significant changes over time in TNF- α and IL-6 levels ($P = 0.0014$ and $P = 0.00024$ respectively) (Fig. 1A and B). No significant changes over time were observed in IL-4 and IL-12 levels (Fig. 1C and D). The more pronounced effect (i.e. increase in IL-6 (Wilcoxon matched pairs test, $P = 0.0015$) and TNF- α levels ($P = 0.0015$)) was observed at 6 h following the first administration of hGH (Fig. 1A and B). We could not find a statistically significant correlation between cytokine increase at 6 h after the first injection of hGH and the increase in IGF-I levels after 3 months of treatment (IL-6: $r = 0.09$, $P = 0.82$; TNF- α : $r = 0.21$, $P = 0.61$).

Discussion

GH has long been postulated to stimulate immune and inflammatory reactions. It is reasonable to hypothesize that, whether GH can influence the immune system, its absence should lead to alterations in the immune response. However, the results of the studies in animals and in humans are conflicting. The dwarf Snell mice,

Table 1 Basal serum levels of the cytokines in GHD children and healthy control children. Data are shown as medians and 25th–75th percentile. The significance levels (P) of the differences between GHD children and healthy controls are shown.

Cytokine	GHD children	Healthy children	P
IL-4 (pg/ml)	23.9 (22.3–37.5)	23.4 (19.9–27.7)	0.662
IL-6 (pg/ml)	5.6 (0.5–15.8)	0.6 (0.2–60.1)	0.938
IL-12 (pg/ml)	5.3 (2–54.6)	6.2 (3–31.2)	0.898
TNF- α (pg/ml)	6.1 (1–124.6)	9 (2.8–1063.7)	0.228

Figure 1 Circulating levels of cytokines (median and 25th–75th percentile) in GHD children before and after 6 and 24 h, and then after 3 months (90 d) of GH treatment and in healthy controls (CTRL). * $P = 0.0015$, Wilcoxon matched pairs test.

having both a congenital hypopituitarism and severe cell-mediated as well as humoral immunodeficiency, normalize their immune function after GH treatment (7, 8). Moreover, experimental administration of anti-serum against GH leads to the suppression of antibody formation in mice, confirming the effect of GH on immune function (9). Hypophysectomized rats have markedly depressed synthesis of TNF- α by macrophages and exogenous GH partially reverses the effect of hypophysectomy, supporting the hypothesis that the pituitary gland is needed for the synthesis of TNF- α by macrophages (10, 11). In contrast, no clinical signs of immune dysfunction have been observed in humans in spite of severe deficiency due to either a mutation in the Pit-1 gene or a deletion of the GH-N gene. In fact, GH deficiency is not usually associated with immunodeficiency, except for rare X-linked combinations of isolated GH deficiency and agammaglobulinaemia (12). Although GHD patients have no increased susceptibility to infections, some immune parameters may be impaired in these subjects (13, 14).

In agreement with the absence of an overt immune defect in children with GHD, our data showed that cytokine basal levels in GHD patients do not differ from those of healthy children. On the contrary, we found a significant increase in serum IL-6 and TNF- α values

in GHD patients 6 h after an acute hGH administration. The levels of the cytokines started to decrease 24 h after the injection and remain unchanged for the following 3 months. Our results suggest that GH administration has a deep impact on cytokine synthesis in children with complete or partial GH deficiency. The increase in TNF- α levels confirmed our previous findings that demonstrated high cytokine levels 6 h after hGH injection in GHD children (5) and after the 4th hGH injection in short non-GHD children (6).

Furthermore, *in vitro* studies demonstrate that the presence of GH increases the production of pro-inflammatory cytokines by monocytes in response to lipopolysaccharide (15). TNF- α and IL-6 are cytokines produced particularly by monocytes/macrophages and their increase after GH administration could be due to the well-known macrophage-activating potential of GH itself (16). This effect could be direct or mediated by GH-dependent factors, such as IGF-I because immune cells express both GH and IGF-I receptors (1). However, the fact that we observed an increase in IL-6 and TNF- α levels, in particular 6 h following the first administration of hGH, suggests a rather prompt effect on the macrophage. It is conceivable to hypothesize that this may reflect a direct effect of GH on macrophage, rather than an effect mediated by IGF-I.

Indeed, on the other hand, GH therapy does not seem to affect serum IL-4 and IL-12 levels during the treatment. IL-4 is known to promote the immune cell differentiation toward the Th2 phenotype and to transform naive Th cells into IL-4-producing T cells; Th2 cytokines promote Th2 activities and inhibit Th1 activities and vice versa. Takagi *et al.* (17) demonstrated that in burned mice, where there is a conversion of Th cell populations from Th1 cells to Th2 cells, administration of recombinant GH leads to a Th1-dominant response, supporting the role of GH in modulating the release of type 1 cytokines. These data are in accordance with our results of no effect of GH therapy on serum IL-4 levels. Furthermore, other studies have demonstrated that, in particular non-pathologic conditions, high levels of serum GH are not necessarily correlated with elevated serum IL-4 concentrations (18, 19). Unlike IL-4, IL-12 is a cytokine that stimulates a switch toward Th1 cells with production of type 1 cytokines; nevertheless, GH administration did not alter serum levels of this cytokine at any time after the beginning of the therapy. These data are in apparent contrast with our previous results that showed a stimulating effect of GH injection on IL-12 release in non-GHD children after hGH administration, suggesting that low endogenous GH levels could alter cytokine response. Furthermore, the ELISA we used detected only the p70 form of IL-12 and, it is possible, an increase in the p40 subunit, whose production greatly exceeds the p70 one, and is easily detectable with an ELISA method. In contrast to our findings in children, in GHD adults basal serum TNF- α and IL6 levels are higher than in controls and decrease after prolonged GH administration, suggesting a different regulation of cytokine production in adulthood (20).

In conclusion, we have demonstrated an effect of hGH administration on the *in vivo* levels of the pro-inflammatory cytokine IL-6 and TNF- α . Despite this action of GH, in GHD children a normal immune function persists.

References

- Kooijman R, Willems M, De Haas CJ, Rijkers GT, Schuurmans AL, van Buul-Offers SC, Heijnen CJ & Zegers BJ. Expression of type I insulin-like growth factor receptors on human peripheral blood mononuclear cells. *Endocrinology* 1992 **131** 2244–2250.
- Rapaport R, Sills IN, Green L, Barrett P, Labus J, Skuza KA, Chartoff A, Goode L, Stene M & Petersen BH. Detection of human growth hormone receptors on IM-9 cells and peripheral blood mononuclear cell subsets by flow cytometry: correlation with growth hormone-binding protein levels. *Journal of Clinical Endocrinology and Metabolism* 1995 **80** 2612–2619.
- Berczi I. The effects of growth hormone and related hormones on the immune system. In *Pituitary Function and Immunity*, pp 133–159. Ed. I Berczi. Boca Raton, FL: CRC Press, 1986.
- Clark R. The somatogenic hormones and insulin-like growth factor-1: stimulators of lymphopoiesis and immune function. *Endocrine Reviews* 1997 **18** 157–179.
- Bozzola M, De Amici M, Zecca M, Schimpff RM & Rapaport R. Modulating effect of human growth hormone on tumor necrosis factor- α and interleukin-1 β . *European Journal of Endocrinology* 1998 **138** 640–643.
- Bozzola M, De Benedetti F, De Amici M, Jouret B, Travaglini P, Pagani S, Conte F & Tauber M. Stimulating effect of growth hormone on cytokine release in children. *European Journal of Endocrinology* 2003 **149** 1–5.
- Pierpaoli W, Baroni C, Fabris N & Sorkin E. Hormones and immunological capacity. II. Reconstitution of antibody production in hormonally deficient mice by somatotrophic hormone, thyrotrophic hormone and thyroxine. *Immunology* 1969 **16** 217–230.
- Berczi I. Pituitary hormones and immune function. *Acta Paediatrica* 1997 **423** (Suppl) 70–75.
- Pierpaoli W & Sorkin E. Hormones and immunological capacity. I. Effect of heterologous anti-growth hormone antiserum on thymus and peripheral lymphatic tissue in mice. Induction of a wasting syndrome. *Journal of Immunology* 1968 **101** 1036–1043.
- Edwards CK III, Lorence RM, Dunham DM, Arkins S, Yunker LM, Greager JA, Walter RJ, Dantzer R & Kelley KW. Hypophysectomy inhibits the synthesis of tumor necrosis factor alpha by rat macrophages: partial restoration by exogenous growth hormone or interferon gamma. *Endocrinology* 1991 **128** 989–996.
- de Kossado S, Grau GE, Daneva T, Pointaire P, Fossati L, Ody C, Zapf J, Piguet PF, Gaillard RC & Vassalli P. Tumor necrosis factor α is involved in mouse growth and lymphoid tissue development. *Journal of Experimental Medicine* 1992 **176** 1259–1264.
- Fleisher TA, White RM, Broder S, Nissley SP, Blaese RM, Mulvihill JJ, Olive G & Waldmann TA. X-linked hypogammaglobulinemia and isolated growth hormone deficiency. *New England Journal of Medicine* 1980 **302** 1429–1434.
- Kelley KW. The role of growth hormone in modulation of the immune response. *Annals of the New York Academy of Sciences* 1990 **594** 95–103.
- Bozzola M, Valtorta A, Moretta A, Cisternino M, Biscaldi I & Schimpff RM. *In vitro* and *in vivo* effect of growth hormone on cytotoxic activity. *Journal of Pediatrics* 1990 **117** 596–599.
- Uronen-Hansson H, Allen ML, Lichtarowicz-Krynska E, Aynsley-Green A, Cole TJ, Hoiden-Guthenberg I, Fryklund L & Klein N. Growth hormone enhances proinflammatory cytokine production by monocytes in whole blood. *Growth Hormone & IGF Research* 2003 **13** 282–286.
- Warwick-Davies J, Lowrie DB & Cole PJ. Growth hormone is a human macrophage activating factor. Priming of human monocyte for enhanced release of H2O2. *Journal of Immunology* 1995 **154** 1909–1918.
- Takagi K, Suzuki F, Barrow RE, Wolf SE & Herndon DN. Recombinant human growth hormone modulates Th1 and Th2 cytokine response in burned mice. *Annals of Surgery* 1998 **228** 106–111.
- Cioffi M, Esposito K, Vietri MT, Gaggero P, D'Auria A, Ardovino I, Puca GA & Molinari AM. Cytokine pattern in postmenopause. *Maturitas* 2002 **41** 187–192.
- Suzuki K, Yamada M, Kurakake S, Okamura N, Yamaya K, Liu Q, Kudoh S, Kowatari K, Nakaji S & Sugawara K. Circulating cytokines and hormones with immunosuppressive neutrophil-priming potentials rise after endurance exercise in humans. *European Journal of Applied Physiology* 2000 **81** 281–287.
- Serri O, St-Jacques P, Sartippour M & Renier G. Alterations of monocyte function in patients with growth hormone (GH) deficiency: effect of substitutive GH therapy. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 58–63.

Received 14 April 2004

Accepted 8 October 2004