IMMUNE SYSTEM IN ADULTS WITH CHILDHOOD-ONSET GROWTH HORMONE DEFICIENCY: EFFECT OF GROWTH HORMONE THERAPY

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Objective. To investigate the impact of growth hormone (GH) therapy in adults with childhood-onset GH deficiency on immune system.

Methods. Ten young GH deficient adults (7 males, age 19-28 years) were treated with recombinant human growth hormone for 6 months. The starting dose was 0.5 IU/m^2/day (2 weeks), then it was doubled to 1.0 IU/m^2/day. In 5/10 patients, the dose was further increased to 1.5 IU/m^2/day at 4 weeks of therapy. Immunological studies were performed before treatment and after 6 weeks, 3 months and 6 months and included humoral (IgG, IgA, IgM, C3, C4 and immune complexes) and cellular parameters (total lymphocyte count and counts of CD3+, CD4+, CD8+ and CD19+ lymphocytes, the CD4+/CD8+ ratio and percentage of CD16+56+ and CD3+DR+).

Results. The cellular responses to GH therapy were subtle, but detectable, with the trend to the higher CD4+ and lower CD8+ lymhocytes and maximal changes at 6 months of therapy. They were reflected in CD4/CD8 ratio, which increased from 1.15±0.10 (mean ± S.E.; baseline) to 1.37±0.11 (6 weeks; $P<0.05$), 1.24±0.10 (3 months; n.s.) and to 1.59±0.20 (6 months; $P<0.05$). The response in humoral immunity was characterized by a rapid decrease of circulating immunoglobulins (IgA: 1.40±0.25 g/l [mean±S.E.], baseline; 1.12±0.19, at 6 weeks; $P<0.05$) and C4 (0.25±0.02 g/l, baseline; 0.19±0.01, at 6 weeks; $P<0.05$) and a tendency to an increase in circulating immune complexes (29.1±8.1, baseline; 40.3±7.2, at 6 weeks; n.s.). These observations suggest a temporary immune complex formation after the onset of GH treatment which might play a partial role in developing edema as a side effect of GH treatment, besides the known effect of GH on water retention.

Conclusions. GH therapy in GH deficient young adults has a measurable effect on the increase of CD4/CD8 ratio and on the formation of immune complexes.

Key words: Growth hormone (GH) – GH deficiency – GH therapy – Adults – Immune system – Lymphocytes – Immunoglobulins – Immune complexes

Growth hormone (GH) is a neuroendocrine hormone that exerts, besides its well known effect on longitudinal growth, generalized effects on protein, lipid and carbohydrate metabolism, as summarized by Strobl and Thomas (1994). Several authors (Strobl and Thomas 1994; Murphy et al. 1995; Auernhammer and Strasburger 1995; Van Buul-Offers and Kooijman 1998) reported, that GH has some additional roles, including the numerous effects on the regulation, function and development of the immune system. Numerous studies showed the effects of GH and consequently the effects mediated

by insulin-like growth factor-I (IGF-I) released upon GH stimulation. Animal models include these of Snell dwarf mice, with congenital GH deficiency combined with thyroid-stimulating hormone (TSH) and prolactin deficiencies due to the mutation in the Pit-1 gene, which present with an arrested thymus development and deficient cell mediated immune reactions, as observed by Van Buul-Offers et al. (1978) and Li et al. (1990). Pierpaoli et al. (1969) found that GH has the potential to reverse these changes. The role of GH and the distribution of GH receptors on the immunocompetent cells was studied also in normal mice without GH deficiency. GH receptors were found to be widely distributed in murine lymphoid tissues including central immune organs (thymus and bone marrow), peripheral lymphoid tissues and circulating immunocompetent cells, suggesting complex effect of GH on immune functions, as observed by Dardenne et al. (1998).

The effect of GH on human immune system was the subject of several studies (Abbassi and Bellanti 1985; Rappaport et al. 1986; Bozzola et al. 1988; Manfredi et al. 1994; Strobl and Thomas 1994); Auernhammer and Strasburger 1995; Span et al. 1996; Geffner 1997; Van Buul-Offers and Kooijman 1998; Lebl et al. 1999). However, despite the proven laboratory effect of GH on immune function, no clinical signs of immune defect were found in GH deficient patients, as summarized by Auernhammer and Strasburger (1995). The consequences of GH treatment on the immune parameters were studied in GH deficient children and adults. Several authors (Abbassi and Bellanti 1985; Rappaport et al. 1986; Bozzola et al. 1988; Manfredi et al. 1994) reported contradictory data with no definitive demonstrable effect on immune parameters.

Recently, due to its influence on the immune function, GH treatment attracts the attention for the potential use in GH sufficient patients showing some impairments the treatment of which by GH might be of potential benefit. Nguyen et al. (1998) and Chappel (1999) found that GH treatment in AIDS patients reversed the AIDS-related wasting and improved the general well-being of the subjects due to increased proteoanabolism but with no significant changes particularly in their low CD4 counts. We recently published the data on GH treatment of children after renal transplantation (Lebl et al. 1999), in which we demonstrated subtle changes in cellular immunity.

The aim of this study was to focus our attention on a group of GH deficient adults whose GH therapy was started for its metabolic effects.

Subjects and Methods

Patients. We studied 10 young adult patients (7 males) with childhood onset growth hormone deficiency aged 19-28 years (median 25 years). All of them were diagnosed to be GH deficient before the onset of puberty and all have been treated with pituitary-derived and/or recombinant human GH since the age at diagnosis until they reached their adult height. All of the subjects studied had a multiple pituitary hormone deficiency including TSH deficiency in all 10, gonadotropin deficiency in 6/10 and ACTH deficiency in 2/10. In two of them hypopituitarism was a consequence of a craniopharyngioma, in one it was related to septo-optic dysplasia and in the remaining 7 it was classified as being idiopathic according to normal findings at a CT scan and/or MRI.

The diagnosis of GH deficiency was reassured using an insulin tolerance test before the onset of this study. In all subjects, the stimulated GH level did not exceed 3 ng/ml. All concommitant hormonal defects were properly substituted with L-thyroxine and/or hydrocortisone and/or sexual steroids at the GH testing as well as during the entire study period. No changes in this treatment were done for at least 3 months before the study started.

One patient had to be excluded from the evaluation of humoral immunity because of insufficient baseline data.

Study design. The patients were treated with recombinant human GH (Novo Nordisk, Denmark) given sc. daily in the evening. All patients started with a dose 0.5 IU/m^2/day for 2 weeks, then the dose was doubled to 1.0 IU/m^2/day. In 5/10 patients, it was further increased to 1.5 IU/m^2/day at 4 weeks of therapy. Immune parameters were estimated before treatment and after 6 weeks, 3 months and 6 months of therapy with GH. The estimation included humoral parameters (IgG, IgA, IgM, C3, C4 and immune complexes) as well as cellular parameters (total lymphocyte count and the counts of CD3+, CD4+, CD8+ and CD19+ lymphocytes as well as the CD4+/CD8+

ratio and percentage of CD16+56+ and CD3+DR+ lymphocytes). All parameters of humoral immunity were measured by nephelometry with appropriate antisera (Behring, Marburg, Germany). Absolute numbers of CD3+, CD4+, CD8+ and CD19+ expressing lymphocytes were measured on Epics Profile system. The fluorescein and phycoerythin labelled monoclonal antibodies were purchased from Immunotech (Marseille, France). IGF-I levels were estimated as total IGF-I before therapy and after 3 and 6 months of therapy with GH, using the IRMA kit (Immunotech, Marseille, France).

Statistical evaluation. Statistical evaluation was performed using Student's t-test for paired data and P values were adjusted for multiple comparisons.

Ethics. The study protocol was approved by the Ethical Committee of the University Hospital Prague-Motol. All subjects obtained written information and gave their consent to participate in the study.

Results

The results of immunological tests before and during therapy with GH are summarized in detail in Tab. 1 and 2. IGF increased from the very low baseline levels of 35±2 ng/ml (mean±S.E.) to 232±25 ng/ml (at 3 months of GH administration; P<0.0001) and to 249±35 ng/ml (at 6 months of GH administration; P<0.0001) which both represent low normal values according to age.

Three out of ten patients developed transient joint pain or overt oedema of the joints within the first 2-4 weeks of GH therapy, but the treatment schedule was not changed. These symptoms resolved spontaneously during further 1-2 weeks of GH administration.

Tab. 1. Lymphocyte subsets in ten GH deficient adults before and during therapy with GH (data as mean ± S.E.).

Duration of treatment	before	6 weeks	3 months	6 months
Leukocytes ($x10^9$/L)	6.30±0.31	6.39±0.76	5.96±0.41	6.11±0.34
Lymphocytes ($x10^9$/L)	2.11±0.15	1.95±0.21	2.01±0.19	2.01±0.22
CD3+ ($x10^9$/L)	1.51±0.11	1.41±0.15	1.44±0.14	1.52±0.16
CD4+ ($x10^9$/L)	0.80±0.07	0.80±0.09	0.79±0.06	0.91±0.11
CD8+ ($x10^9$/L)	0.73±0.08	0.61±0.08	0.69±0.10	0.60±0.07
CD4+/CD8+	1.15±0.10	1.37±0.11 *	1.24±0.10	1.59±0.20 *
CD19+ ($x10^9$/L)	0.34±0.07	0.31±0.07	0.36±0.07	0.31±0.08
CD16+56+ (%)	11.6±1.8	12.0±1.7	10.4±1.7	10.8±1.8
CD3+DR+ (%)	5.90±1.39	3.89±0.75	5.20±1.14	4.17±0.73

* P<0.05 vs. before treatment; corrected for multiple analyses

Tab. 2. Parameters of humoral immunity in nine GH deficient adults before and during therapy with GH (data as mean ± S.E.).

Duration of treatment	before	6 weeks	3 months	6 months
IgG (g/L)	14.4±0.9	13.2±0.6	13.8±0.7	14.7±0.7
IgA (g/L)	1.40±0.25	1.12±0.19 *	1.13±0.19 *	1.25±0.21
IgM (g/L)	2.03±0.30	1.75±0.29	1.96±0.30	2.07±0.30
C3 (g/L)	0.77±0.04	0.79±0.03	0.78±0.04	0.69±0.04
C4 (g/L)	0.25±0.02	0.19±0.01 *	0.23±0.01	0.23±0.01
immune complexes	29.1±8.1	40.3±7.2	35.4±3.4	18.4±2.5

* P<0.05 vs. before treatment; corrected for multiple analyses

Discussion

Despite the numerous recently recognized effects of GH on the immune system (Auernhammer and Strasburger 1995; Geffner 1997; Van Buul-Offers and Kooijman 1998), the investigation of the immune functions is not in the primary interest of the studies on GH treatment in GH deficient subjects, as these patients do not present with any clinical signs of immune disorder. With the growing knowledge on the mechanisms connecting the endocrine and immune systems and recognized role of GH and consequently IGF-I in these interactions, the attention is being directed to the detailed studies of GH influence on the immune functions. Both GH and IGF-I are synthesized and secreted by various immunocompetent cells and, in addition, the receptors for GH and IGF-I are expressed on immune cells and organs (Auernhammer and Strasburger 1995). Both substances have the direct and complex influence on the immune cells in the periphery, but they have as well a profound effect on central immune organs (e.g. bone marrow and thymus), as summarized by Geffner (1997). Very recently, Kelley et al. (1998) and Burgess et al. (1999) elucidated in more detail the mechanisms of IGF-I mediated ac-

tion on hematopoietic progenitor cells and thymus and consequently on the immune status of the patients. According to these results, IGF-I inhibits the apoptosis of hematopoietic progenitors of the myeloid and lymphoid lineages and promotes their replication and survival. Moreover, the function of IGF-I exerts a similar function as interleukin-4 in the induction of the immune response (Burgess et al. 1999).

As more studies bring the clear evidence on important GH influence on the immune system, we decided to monitor immune parameters in a group of adult GH deficient patients treated with GH. These patients were regularly treated in childhood and reached average final height. Current GH therapy was established with the aim to improve the metabolic status of the patients. At the beginning of the study, the whole group showed remarkably normal immune parameters of both humoral and cellular branches of the immune system. The cellular responses to the GH therapy were subtle, but detectable, with the trend to the higher CD4 and lower CD8 positive lymhocytes during the therapy with maximal changes observed in the 6 months' period. These changes are reflected in CD4/CD8 ratio, which continued to increase over the follow up period and reached statistical significance at 6 weeks as well as at 6 months of treatment. We observed the same trend of the changes in cellular immunity in a group of children treated with GH after renal transplantation (Lebl et al. 1999). Despite the different background in these two groups, e.g. children with normal GH secretion treated with GH with the aim to improve their growth impaired due to the chronic renal insufficiency versus GH deficient adults – GH administration exerts a similar effect on the observed parameters. The effect was more profound in the group of children which may reflect the higher GH dose given. The exact mode of action of GH and/or IGF-I is still unclear and in the light of the recent studies by Kelley et al. (1998) and Burgess et al. (1999) requires further, more detailed and functional studies, including the cytokine profile and Th1xTh2 balance in the population of growing CD4 cells after GH therapy.

Besides changes in cellular immunity, we found changes in the parameters of humoral immunity, notably the dynamic movement in the levels of immunoglobulins, complement and circulating immune complexes. The decrease of immunoglobulins, mainly IgA and a simultaneous decrease of C4 and increase of circulating immune complexes suggests a temporary immune complex formation in the first six weeks of treatment with return to normal baseline at 3 and 6 months. The time coincidence of detected immune complexes with developing edema as a side effect of GH treatment invites the question of the partial role of the immune system in edema formation, besides the known effect of GH on water retention.

We conclude that in our two consequent studies of the effect of GH on the immune parameters, in children following renal transplantation (Lebl et al. 1999) as well as in GH deficient adults, we consistently observed minor increase of CD4 lymphocytes with similar decrease of CD8 positive cells, which led to the subtle, but statistically significant increase of CD4/CD8 ratio. In the current study, we also observed the temporary formation of immune complexes. GH has thus a measurable effect on the immune parameters. However, the precise mode of action of GH on the immune system requires further fine and detailed studies.

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BOOK REVIEW

THYROID CANCER
A COMPREHENSIVE GUIDE TO CLINICAL MANAGEMENT

EDITED BY LEONARD WARTOFSKY (WASHINGTON, DC)

HUMANA PRESS (TOTOWA, NEW JERSEY) 1999
E-MAIL: HUMANA@HUMANAPR.COM, 515 PAGES, HARD COVER US $ 175.00,

"It sometimes seems that thyroid carcinoma is a neglected orphan among human cancers, which is at the root of some important issues. Thyroid carcinomas comprise a diverse group of malignancies ranging from indolent microscopic papillary carcinomas that pose no treat to survival to anaplastic carcinomas that are the most vicious carcinomas afflicting humans. Yet, because of its low incidence, there have been no prospective randomized clinical trials of the treatment of thyroid carcinoma." This is a fragment of the "Foreword" written by outstanding thyroid surgeon Ernest L. Mazaferri.

This comprehensive book brings an instructive review of present knowledge on the pathology, etiology, epidemiology, diagnostic methods and the methods of surgical, radioiodine and chemotherapeutic treatment of various forms of thyroid cancer. Written by outstanding experts and professionaly edited by Leonard Wartofsky, it may be considered of substantial utility to all physicians dealing with thyroid diseases.

A total of 52 comprehensive chapters is divided into 9 sections dealing with the diagnostics and management of thyroid nodule (Part I), general considerations on the thyroid cancer (Part II), clinical aspects, pathology, treatment, follow-up, prognosis and special aspects in children of papillary carcinoma (Part III), the same aspects of follicular (Part IV) and anaplastic carcinoma (Part V) and lymphoma (part VI). Next sections are devoted to medullary carcinoma (Part VII), unusual thyroid cancers (Part VIII) and future directions (Part IX). A number of up to date references and instructive tables, figures and photos are attached to each chapter. In addition to basal theoretical knowledge on each problem discussed in individual chapters, there is a number of practical and handy instructions such as a detailed description of fine needle aspiration technique, detailed descriptions of external irradiation techniques and doses, sonography, various imaging procedures and strategies of follow-up the patients after the surgical and radiation treatment. However, some actual questions on the extent of thyroidectomy such as lobectomy versus subtotal or ner-total thyroidectomy would perhaps deserve more detailed discussion.

Of special value may be considered the chapters on molecular pathology of thyroid cancer including significant recent achievements of molecular genetic analysis of inheritance pattern in families with medullary carcinoma.

Finaly, again few words of Ernest L. Mazaferri: *"I believe the knowledge contained in Thyroid Cancer will give the practicing clinicians the necessary information to provide patients the latest and best diagnostic and therapeutic techniques."*

Pavel Langer