LYMPHOCYTE SUBSETS IN RENAL TRANSPLANT RECIPIENTS DURING GROWTH HORMONE THERAPY

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Objective. To evaluate the effect of growth hormone treatment on growth, levels of insulin-like growth factor I (IGF-I) and lymphocyte subsets in immunosuppressed renal allograft recipients.

Methods. 18 children (aged 8.0-16.6 years) received growth hormone 1 IU/Kg/week daily for two years. Height, IGF-I levels and in 11/18 patients, lymphocyte subsets were evaluated serially.

Results. Standardized growth velocity increased from -1.0±1.5 to +1.2±2.2 and standardized IGF-I levels from +0.8±1.5 to +3.1±1.1 (1 year) and to +1.4±1.7 (2 years). The total lymphocyte count and the number of T lymphocytes (CD3+) decreased. The decrease was more marked in CD8+ (from 1.5±0.3 $x10^9$/L to 0.9±0.3 $x10^9$/L, 1 year and to 0.8±0.1 $x10^9$/L, 2 years) compared to CD4+ (from 1.5±0.3 $x10^9$/L to 1.0±0.2 $x10^9$/L, 1 year and to 1.3±0.2 $x10^9$/L, 2 years), resulting in an increment of the CD4+/CD8+ index.

Conclusions. The differential effect of growth hormone treatment on CD4+ and CD8+ lymphocytes might be explained by different expression of the IGF-I receptor in these distinct subsets.

Key words: Growth hormone – Renal transplantation – IGF-I – Lymphocyte subsets – Children

The growth is frequently impaired in children with chronic renal failure, even after a successful kidney transplantation. In addition to the possible continuous effect of the underlying disease and suboptimal kidney function, Prednisone as part of the immunosuppressive therapy may have an adverse effect on growth because of its interference with growth hormone secretion and action as well as with the synthesis of insulin-like growth factor I (IGF-I), as previously suggested by Fine (1994a) and Schaefer et al. (1994).

Since the late 1980s, several studies have been conducted to evaluate the effect of growth hormone treatment in children with renal transplant (Fine et al. 1991, 1994b; Bartosh et al. 1992; Van Dop et al. 1992; Toenshoff et al. 1993). All published data confirm an increased growth velocity in growth hormone recipients, although the final outcome in terms of final height remains unknown. Another topic of prominent interest are safety data of such a treatment, above all the potential risk of the induction of acute or aggravation of chronic rejection crisis.

Growth hormone is known to interact with the immune system and its administration could theoretically impair the sensitive immunological equilibrium induced by posttransplant immunosuppressive therapy, as previously suggested by Wit et al. (1993) and Giustina et al. (1995). Several authors (Fine et al. 1991 Bartosh et al. 1992; Van Dop et al. 1992; Toenshoff et al. 1993) reported sporadic rejection episodes in patients treated with growth hormone in open-labeled studies, but today no conclusion about the risk of rejection can be drawn. Interestingly, data on immune parameters per se were not followed in any of the studies already published.

In our study, we are presenting data on 2 years of growth hormone treatment in 18 children after renal transplantation. Investigation of cellular immunity

T a b l e 1
The underlying kidney disease in 18 children after renal transplantation

	n
Juvenile nephronophtisis	6
Chronic glomerulonephritis	5
Cystinosis	2
Congenital kidney dysplasia	1
Polycystic kidney disease	1
Obstructive uropathy	1
Haemolytic-uremic syndrome	1
Toxic effect of treatment with Cis-Platine	1

was provided during growth hormone therapy in a substantial proportion of our patients.

Subjects and Methods

Patients. 18 children (13 boys) aged 8.0 – 16.6 years (median, 13.6) entered the study. Their bone age estimated by the method described by TANNER et al. (1975) was 5.4 – 15.3 years (median, 10.1) and the interval between renal transplantation and onset of growth hormone treatment was 0.5 – 7.5 years (median, 2.8). The underlying kidney diseases are listed in Tab. 1. Other relevant patients' data are summarized in Tab. 2, first column.

All children were immunosuppressed by the combination of Cyclosporine A, Prednisone and in 12/18 patients, with additional Azathioprine. The serum Cyclosporine A levels in these patients (as determined by HPLC) ranged between 75.8 and 131.0 ng/ml (mean, 102.5±14.5) within the study period and their Prednisone dose varied between 0.08-0.27 mg/kg/day (mean, 0.15±0.06).

Study design. Patients were treated with recombinant human growth hormone (1 IU/Kg/week, given every day s.c. before going to bed). The dose of growth hormone was adjusted to the actual body weight every 3 months. In this study the data after 2 years were analyzed. Patients were seen in the outpatient clinic every three months for clinical examination. Body height was measured with a Harpenden stadiometer, the mean of three consecutive measurements, and pubertal status was recorded on each occasion. Height and growth velocity were related to chronological age and were expressed in cm and standard deviation score (SDS) according to standards established by PRADER et al. (1989). Laboratory control included basic haematological and biochemical parameters, estimation of renal allograft function, thyroid status and serum IGF-I level.

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

IGF-I levels. Serum IGF-I was determined by radioimmunoassay after acid-ethanol extraction. IGF-I rabbit antiserum (UB3-189) was from the Hormone Distribution Programme of NIDDK, USA, and was used at a final dilution of 1:5000. The International Reference Preparation IGF-I 87/518 was from NIBSC, UK and was used as standard. The tracer, prepared by iodination of recombinant IGF-I and purified by HPLC, was from Amersham. The detection limit of the assay was 0.1 ng/tube. The intra-assay CV at 35 % binding was 6.1 %.

Lymphocyte subsets. The analysis of lymphocytes was repeatedly performed in 11 patients during the regular follow-up periods. Percentage and absolute numbers of CD3+, CD4+, CD8+ and CD20+ expressing lymphocytes were measured on Epics Profile system. The fluorescein and phycoerythrin labeled monoclonal antibodies were purchased from Immunotech, Marseille, France.

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

Results

Drop-out. The data of all patients were complete during the first year of the study. 6 subjects finished the study in the second year for various reasons: Two patients developed a chronic graft rejection, in two patients the family and physician decided to terminate therapy because of poor growth velocity and in two cases the patient and/or the family decided to terminate treatment for private reasons. No other adverse events of clinical or biochemical parameters were observed.

Renal function. The mean glomerular filtration rate did not change within the study period (Tab. 2).

Table 2
Height, growth velocity, bone age progression, glomerular filtration rate and IGF-I levels during the study period (data as mean ± SD)

Month of study	0	6	12	18	24
number (prepub./pub.)	8/10	5/13	3/15	2/13	0/12
Height (SDS)	-3.5±1.6	-3.2±1.7***	-3.1±1.6**	-3.0±1.7*	-2.5±1.2*
GV (cm/year)	3.9±2.4[#]	-	7.9±2.8***	-	5.6±2.4
GV (SDS)	-1.0±1.5[#]	-	+1.2±2.2***	-	+1.2±2.2**
BA/CA per year	-	-	1.7±1.2*	-	1.5±0.8
GFR[##] (ml/sec/1.73 m^2)	1.15±0.47	-	1.10±0.57	-	1.00±0.57
IGF-I (SDS)	0.8±1.5	3.2±1.2**	3.1±1.1**	2.1±1.0*	1.4±1.7

* P<0.05, ** P<0.01, *** P<0.001 vs. 0 months; in BA/CA, vs. value 1.0
[#] Calculated from two measurements taken in distance of 11 months (median; range 2-13 months) prior to study
[##] Estimated according to the Schwartz formula
BA, Bone age
CA, Chronological age
GFR, Glomerular filtration rate
GV, Growth velocity
IGF-I, Insulin-like growth factor I
SDS, Standard deviation score

T a b l e 3
Lymphocyte subsets in 11 immunosuppressed children with renal transplant before and during therapy with growth hormone (data as mean±SEM)

Month of study	0	3	6	12	24
n	11	8	8	10	7
Lymphocytes (x10^9/L)	3.7±0.5	2.6±0.3*	2.6±0.5*	2.9±0.3*	2.5±0.3
CD3+ (x10^9/L)	3.1±0.4	2.2±0.2	2.1±0.5*	2.3±0.2	2.1±0.3
CD4+ (x10^9/L)	1.5±0.3	1.0±0.1*	1.0±0.2	1.3±0.1	1.3±0.2
CD8+ (x10^9/L)	1.5±0.3	1.0±0.2	0.9±0.3**	1.0±0.1	0.8±0.1
CD4+/CD8+	1.1±0.1	1.2±0.2	1.4±0.2	1.4±0.1*	1.7±0.2
CD20+ (x10^9/L)	0.3±0.1	0.1±0.0	0.2±0.1	0.2±0.0	0.1±0.0

* P<0.05, ** P<0.01 vs. 0 months

Bone age. Bone age progression (bone age/chronological age per year) was slightly advanced during growth hormone therapy (Tab. 2).

Growth velocity and statural height. Growth velocity increased significantly during the first study year (Tab. 2). During the second year of study, growth rate in cm/year decreased but remained high compared to chronological age.

IGF-I. Serum IGF-I levels (expressed in SDS for chronological age) increased significantly after initiation of growth hormone therapy and remained elevated during the whole first year of study. Within the second year, the elevation of IGF-I levels was less pronounced (Tab. 2).

Lymphocyte subsets. All patients but one from Prague center (n = 11) were included in the immunological study. The results of one excluded patient were not considered to be relevant, because his disease was complicated with persistent leukopenia.

Results of immunological investigations are summarized in Tab 3. The total lymphocyte count and the number of T lymphocytes (CD3+) decreased during growth hormone therapy. Among T lymphocyte subsets, the decrease was more marked in CD8+ lymphocytes com-

pared to CD4+ lymphocytes, resulting in an increase of the CD4+/CD8+ index. This shift in numbers of investigated lymphocytes started within the first 3 months of growth hormone administration and remained relatively stable during the whole study period.

Discussion

Growth hormone is a neuroendocrine hormone that exerts numerous effects on function and development of the immune system. This has been proven by in vitro studies and in animal models and summarized by Murphy et al. (1995). Badolato et al. (1994) found that receptors for growth hormone are expressed on human lymphocytes. Animals with severe congenital growth hormone deficiency combined with thyroid-stimulating hormone and prolactin deficiencies due to a mutation in the Pit-1 gene, the Snell dwarf mice, have an arrested thymus development and deficient cell-mediated immune reaction, as observed by Van Buul-Offers et al. (1978).

In humans, growth hormone deficiency does usually not exert any clinical signs of altered immunity. In clinical studies conducted by Abbassi and Bellanti (1985), Rapaport et al. (1986) and Bozzola et al. (1988), the results of immunological investigations were mostly in the expected normal range. Only slight derangements have been found rather in the granulocyte than in the lymphocyte functions by Manfredi et al. (1994). Kotzmann et al. (1994) studied the effect of supraphysiological growth hormone levels on the immune system in the model of human acromegaly. In these patients, they found an enhanced T cell activity, but no change in cell numbers of CD3+, CD4+ or CD8+.

Growth hormone recipients with more or less normal endogenous growth hormone secretion, e.g. girls with Turner syndrome, represent another model for studies of the growth hormone effect on the immune system in vivo. Rongen-Westerlaken et al. (1991) observed, that in these patients the previously reduced number of NK cells returned to normal and in some of them circulating B lymphocytes decreased after treatment with growth hormone.

The more prominent effects of growth hormone on phagocytes or B cells can be partially explained by the fact that growth hormone, besides its own direct influence on immune system, stimulates the production of IGF-I. This substance is lately in the prominent interest of immunologists and has its own marked and now more investigated effect on immune system than growth hormone itself. IGF-I binds to specific receptors, which were detected on the cells of immune system, and the binding capacity for IGF-I was monocytes>B cells>T cells. In spite of low expression of receptors for IGF-I on T lymphocytes this factor has functional influence on both CD4+ and CD8+ lymphocytes, as observed by Xu et al. (1995).

In the last years children after renal transplantation are increasingly treated with growth hormone, but so far no immunological data from these studies are available. In these children, the hormonal as well as the immunological situation is rather complex. On one hand, growth hormone is being administered in order to overcome the growth hormone resistant state caused by metabolic sequelae of chronic renal failure and/or the underlying metabolic disease as well as by the glucocorticoid therapy which interferes with growth hormone production and action. On the other hand, the immune system is altered by continuous systemic immunosuppression, which is vital for the patient.

Direct immunostimulatory effect of growth hormone treatment, if any, could be fatal in these patients. However, in studies published so far, a dramatic increase of rejection crises was not observed (Fine et al. 1991; Bartosh et al. 1992; Van Dop et al. 1992; Toenshoff et al. 1993), but an increased risk cannot be excluded. Giustina et al. (1995) administered growth hormone in a clinical trial to adults on long-term prednisone treatment for a systemic autoimmune disease. They found that growth hormone had the potential to counteract some of the immunosuppressive effects of prednisone therapy. They observed a slight increase of T-helper, a decrease of T-suppressor and a marked increment in T4+/T8+ ratio.

Our observations in growth hormone treated children with renal transplants are compatible with these observations. The total number of lymphocytes decreased but more subtle changes occurred in lymphocyte subsets. B lymphocytes were apparently less affected by growth hormone, whereas a more profound decline of CD8+ compared to CD4+ cells led to an increment in T4+/T8+ ratio.

The precise mechanism, which led to observed changes, is very difficult to elucidate because of the complex interaction of growth hormone and IGF-I with the regulatory mechanisms of the immune system. They share some parts of the receptors with cytokines (e.g. ciliary neurotrophic factor or interleukin-6, as found by PANAYOTATOS et al. 1995) or they use the same intracellular signaling pathways as cytokines with immune functions, as suggested by GOUILLEUX et al. (1995) and KOOIJMAN et al. (1995a). Their effect on the cells of the immune system is proven, but the consequences of the binding of these substances to their receptor are not clear so far because of their extremely complex action. The responsiveness of the immune cells to IGF-I differs markedly according to the maturation and activation stage of the cell, which is probably the reason for the controversial reports about the action of IGF-I (KOOIJMAN et al. 1995a,b). The activation of T lymphocytes requires the expression of interleukin-2 receptor and the binding of interleukin-2, as observed by XU et al. (1995). The effect of growth hormone and consequently of IGF-I in our study is influenced by the immune suppression of the patients with corticosteroids and Cyclosporine A, which both negatively influence the secretion of interleukin-2 and so might change the effect of IGF-I on the lymphocytes. The differential effect on CD4+ and CD8+ lymphocytes might be explained by different expression of the receptor for IGF-I on these distinct subsets.

The unanswered question remains the possible effect of growth hormone and/or IGF-I in the central immune organs, bone marrow and especially thymus. Here the maturation of the lymphocytes is under complex influence of the array of cytokines, with which both growth hormone and IGF-I may interfere from the same reasons of shared receptors and signaling pathways. KOOIJMAN et al. (1995a) found the IGF-I receptors to be present much more abundantly on thymocytes than on peripheral T cells, so the hypothesis of the central immune action or combined central-peripheral immune action of growth hormone and IGF-I cannot be excluded. However, we need further more detailed studies to find out the precise mode of action of growth hormone on immunosuppressed patients in order to better judge between the benefits of improved growth and the possible risks of this treatment.

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