0013-7227/96/$03.00/0
Endocrinology

Vol. 137, No. 12
Printed in U.S.A.

Insulin-Like Growth Factor I Is an Independent Coregulatory Modulator of Natural Killer (NK) Cell Activity*

C. J. AUERNHAMMER, H. FELDMEIER, R. NASS, K. PACHMANN, AND C. J. STRASBURGER

Medizinische Klinik, Klinikum Innenstadt, Ludwig-Maximilians-Universität München, Munich, Germany

ABSTRACT

We aimed to investigate the natural killer (NK) cell activity in hGH-deficient adults and to analyze the effect of insulin-like growth factor (IGF)-I *in vivo* and *in vitro* on NK cell activity. NK cell activity was measured in a 4-h nonisotopic assay with europium-labeled and cryopreserved K-562 cells. NK-cell numbers were measured after incubation with murine monoclonal CD3 and CD16 antibodies by flow cytometry analysis. In a cross-sectional study, the basal and interferon-β (IFN-β) stimulated (1000 IU/ml) NK cell activity of 15 hGH-deficient patients and 15 age- and sex-matched controls was measured. The percentages and absolute numbers of $CD3^-/16^+$ NK-cells were not significantly different in the patient *vs.* control group. The basal and IFN-β stimulated NK cell activity however was significantly decreased in the patient *vs.* control group at all effector/target (E/T) cell ratios from 12.5–100 (*e.g.* 17 ± 3 *vs.* 28 ± 3% lysis without IFN-β, $P < 0.05$, and 42 ± 4 *vs.* 57 ± 4% lysis with IFN-β, $P < 0.05$; both at E/T 50). IGF-I levels of patients and controls showed a significant positive correlation with NK cell activity ($r = 0.37$; $P < 0.05$). In an IGF-I *in vitro* study (IGF-I *in vitro* 250-1250 μg/L), the basal and IFN-β stimulated NK cell activity of 13 hGH-deficient patients and of 18 normal subjects was significantly enhanced by IGF-I *in vitro* (*e.g.* GH-deficient patients: 9 ± 2 *vs.* 10 ± 2% lysis without IFN-β, $P < 0.05$ and 25 ± 4 *vs.* 30 ± 4% lysis with IFN-β, $P < 0.005$; and normal subjects: 15 ± 3 *vs.* 23 ± 3% lysis without IFN-β, $P < 0.001$ and 35 ± 4 *vs.* 44 ± 5% lysis with IFN-β, $P < 0.001$; both at IGF-I 500 μg/L). In summary, in our cross-sectional study, adult GH-deficient patients showed a significantly lower basal and IFN-β stimulated NK cell activity than matched controls, despite equal NK cell numbers. IGF-I levels of patients and controls showed a weak positive correlation with NK cell activity. In an *in vitro* study, IGF-I significantly enhanced basal and IFN-β stimulated NK cell activity of hGH-deficient patients and also of normal subjects. The decreased NK cell activity in GH-deficient patients may be caused at least in part by low serum IGF-I levels. IGF-I appears to be an independent coregulatory modulator of NK cell activity. (*Endocrinology* **137:** 5332–5336, 1996)

RECENTLY, HUMAN GH and insulin-like growth factor(IGF)-I have been shown to play an important role in the cellular and humoral immune system (1–4). hGH and IGF-I receptors have been found on different human lymphocyte subsets including natural killer (NK) cells (5–7). Natural killer cells are $CD3^-CD16^+$ peripheral lymphocytes, mediating non-MHC-restricted cytotoxicity, which can be stimulated by IFN-α, IFN-β, and IL-2 (8–10). Decreased NK cell activity in hGH-deficient children was first observed by Kiess *et al.* (11), and short-term GHRH substitution therapy was reported to have no effect on NK cell activity (12). Other studies also found a decreased NK cell activity in hGH-deficient patients and reported an increase of NK cell activity during hGH substitution therapy (13, 14). *In vivo* IGF-I serum levels were reported to show a weak positive correlation with NK cell activity (14). *In vitro* IGF-I was reported to stimulate basal NK cell activity (6). Recently Watanabe *et al.* (15) have suggested an increased risk of leukemia in GH-deficient children and have hypothesized the reduced NK cell activity in GH-deficiency being one possible explanation for this phenomenon.

We aimed to investigate the effect of hGH-deficiency on NK cell activity in adult patients in comparison to age- and sex-matched controls. After showing a significant correlation between serum levels of IGF-I and NK cell activity, we also aimed to investigate the effects of IGF-I *in vitro* on NK cell activity of hGH-deficient adult patients and normal subjects.

Materials and Methods

Subjects

In the cross-sectional study, 15 hGH-deficient adult patients and 15 age- and sex-matched controls were enrolled. GH-deficiency in the patients was defined by GH serum levels below 2.0 μg/liter during insulin tolerance test or arginin stimulation test and concomitant subnormal IGF-I levels as compared with the age-related reference range. The hGH-deficient patients were of adult (n = 12) or childhood (n = 3) onset and had different etiologies causing their hGH-deficiency: pituitary inactive adenoma (n = 4), prolactinoma (n = 2), craniopharyngeoma (n = 3), septo optic dysplasia (n = 1), Sheehan syndrome (n = 1), and idiopathic isolated childhood onset hGH-deficiency (n = 1), as well as 3 unexplained panhypopituitarisms, suspected to be post-traumatic. In 12 out of 15 of the patients in whom GH-deficiency was combined with impairment of other pituitary functions, adequate substitution therapy was performed with hydrocortisone (n = 8), T_4 (n = 9), sex steroids (n = 12), and vasopressin analogs (n = 3), respectively. Controls were carefully matched for age (37 ± 3 *vs.* 39 ± 3 yr; mean ± SE), sex (5 females and 10 males in each group), and body weight (76 ± 4 *vs.* 76 ± 3 kg) to the patients. The patient group showed significantly lower basal serum

Received May 24, 1996.
Address all correspondence and requests for reprints to: Christian J. Strasburger, M.D., Medizinische Klinik, Klinikum Innenstadt der LMU, Ziemssenstr. 1, 80336 Munich, Germany.
* Parts of this study have been published in abstract form (P2-252) at the 77th Annual Meeting of The Endocrine Society, Washington, DC, 1995 and (P3-25) at the 10th International Congress of Endocrinology, San Francisco, 1996.

levels of GH (0.3 ± 0.1 *vs.* 2.4 ± 0.9 μg/liter; $P < 0.05$), IGF-I (66 ± 12 *vs.* 159 ± 12 μg/liter; $P < 0.001$), and IGFBP-3 (2175 ± 306 *vs.* 3558 ± 184 μg/liter; $P < 0.01$). Serum levels of free T4 and free T3 as well as PRL did not differ significantly between the patients and control group. Blood drawing and determination of NK cell number and NK cell activity were performed on the same day for each patient and matched control subject.

In the IGF-I *in vitro* study, NK cell activity of 13 hGH-deficient patients (sex: 6 female and 7 male; age: 45 ± 4 yr; IGF-I: 40 ± 7 μg/liter) and 18 normal subjects (sex: 9 female and 9 male; age: 48 ± 6 yr; IGF-I: 168 ± 23 μg/liter) was investigated without and with addition of IGF-I *in vitro*. Also in this study, GH-deficiency was defined by the above mentioned criteria.

Blood samples were obtained from patients and controls after written informed consent.

Materials

RPMI 1640 medium with HEPES and fetal calf serum were purchased from GIBCO, Paisely, Scotland. Human AB serum, HEPES, DMSO, and Triton-X 100 were from Sigma, St. Louis, MO. Ficoll separating solution, RPMI 1640 medium without HEPES, glutamine, penicillin/streptomycin and amphotericin B were purchased from Seromed, Berlin, Germany. NaCl, KCl, $MgCl_2$, $CaCl_2$, glucose, and DTPA were purchased from Merck, Darmstadt, Germany. Dextrane sulphate was from Pharmacia, Uppsala, Sweden. Murine anti CD3 and anti CD16 monoclonal antibodies were from Dianova, Hamburg, Germany. The lysis and fixation solution for flow cytometry were from Coulter, Krefeld, Germany. Europium was from Aldrich, Milwaukee, WI. V-shaped 96-well plates were from Greiner, Frickenhausen, Germany. Flat-bottom 96-well plates were from Nunc, Roskilde, Denmark. Recombinant (r)hIGF-I was obtained from GroPep, Adelaide, Australia. Recombinant human interferon-β was from Rentschler, Laupheim, Germany. Europium time-resolved fluorescence was measured in a 1232 DELFIA fluorometer. This fluorometer as well as Europium enhancement solution, which trans-chelates Europium-ions into a highly fluorescent complex, were from Wallac, Turku, Finland. The IGF-I RIA was purchased from Biomerieux, Nürtlingen, Germany. Goat antihuman IGFBP-3 antibodies were from Diagnostic Systems Laboratories, Webster, TX.

Lymphocyte preparation from peripheral blood

Lymphocyte preparation from peripheral blood was performed by the Isopaque-Ficoll technique as described by Boyum (16). Briefly, peripheral whole blood was diluted with assay medium (RPMI 1640 medium with 25 mM HEPES, supplemented with 1% human AB serum, 2 mM glutamine, penicillin/streptomycin and amphotericin B) and layered over Isopaque-Ficoll. After centrifugation at 1200 g for 20 min, the interface region was carefully removed with a pipette. Peripheral blood mononuclear cells (PBMC) were washed twice with assay medium and centrifuged at 600 g for 8 min. PBMC were adjusted to 1×10^7cells/ml, and viability was determined by trypan blue staining and was normally higher than 95%.

Measurement of NK cell numbers

Lymphocyte subset measurement was performed by incubation of 50 μl EDTA blood with 20 μl of FITC- and PE-labeled specific murine monoclonal antibodies against CD3 and CD16 antigens for 30 min. Red blood cell lysis and fixation of the sample was done by an automatic cycler. Flow cytometry scan analysis was performed on an EPICS I (Coulter, Krefeld, Germany). The analysis was routinely performed on the day of blood collection.

Labeling of K-562 cells with europium

NK cell activity was measured in a 4-h nonisotopic assay with europium-labeled and cryopreserved K-562 cells, as recently described by Blomberg *et al.* (17, 18) and Maley *et al.* (19). Briefly, K-562 cells (ATCC no. CCL243) were grown in culture medium (RPMI 1640 medium without HEPES, supplemented with 10% FCS, 2 mM glutamine, penicillin/streptomycin and amphotericin B). For labeling, cells were washed with HEPES buffer (50 mM HEPES, 93 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, pH 7.4). 5×10^7 K-562 cells were incubated with 10 ml of labeling buffer (HEPES buffer containing 100 μM europium, 2 mM DTPA, and 500 μM dextran sulphate) for 12 min at 37 C, while cells were gently suspended every 3 min. Then, 10 ml repairing buffer (HEPES buffer containing 2 mM $CaCl_2$ and 10 mM glucose) were added, and incubation was continued for 3 min. After centrifugation, cells were washed once more with repairing buffer and another four times with RPMI 1640 medium. Cells were resuspended in ice-cold RPMI 1640 medium containing 15% FCS and 10% DMSO, and 5×10^6 to 1×10^7 labeled K-562 cells were aliquoted per vial. The vials were slowly frozen to −80 C with the help of a cryo-box containing isopropanol and then transferred to the liquid nitrogen storage.

Measurement of NK cell activity

For performance of NK cell activity assay, one vial containing europium-labeled and cryopreserved K-562 (Eu-K-562) cells was thawed quickly at 37 C, and cells were washed twice with RPMI 1640 medium without HEPES, supplemented with 1% human AB serum, 2 mM glutamine, penicillin/streptomycin, and amphotericin B. Cells were adjusted to 2×10^5cells/ml, and viability was determined by trypan blue staining and was normally higher than 95%. Viable Eu-K-562 cells were pipetted into 96-well v-shaped plates at 10^4 cells per well. 12.5×10^4, 25×10^4, 50×10^4, and 100×10^4 PBMC were pipetted per well, corresponding to E/T ratios of 12.5, 25, 50, and 100. IFN-β or IGF-I were added in volumes of 10 μl. Incubation was performed in a humidified incubator for 4 h at 37 C and 5% CO2. Then, plates were centrifuged at 400 g for 5 min, and 20 μl of supernatant was transferred to a flat-bottom 96-well plate. 200 μl of enhancement solution was added per well, and plates were incubated for 5 min while gently shaking. Then, time resolved fluorescence was measured in a 1232 Delfia fluorometer. NK cell activity was calculated as % lysis = (maximal release − spontaneous release)/(experimental release − spontaneous release). For the measurement of the spontaneous release, Eu-K-562 cells were incubated without PBMC. For the measurement of the maximal release, Eu-K-562 cells were incubated with 0.5% Triton-X 100. The spontaneous release of europium-labeled and cryopreserved K-562 cells was 38,000 ± 2,000 counts (29 ± 1%), while the maximal release was 129,000 ± 5,000 counts, thus yielding a broad dynamic range of approximately 90,000 counts for reliable measurement of NK cell activity.

Assays of IGF-I and IGFBP-3

IGF-I was measured by a commercial RIA using IGFBP saturation by IGF-II (20). Intra- and interassay coefficients of variation were less than 4.0% and less than 7.0% over the entire assay range, respectively. The reference range of 90–350 μg/liter was established for adults between 25–60 yr of age by calculation of the 5th and 95th percentile of the results from 304 healthy volunteers.

IGFBP-3 was measured by a sandwich-type assay using polyclonal goat antihuman IGFBP-3 antibodies as published previously (21). Intra- and interassay coefficients of variation were both less than 6.0% over the entire assay range, respectively. The reference range of 2000–5200 μg/liter was established for adults between 25–60 yr of age by calculation of the 5th and 95th percentile of the results from 295 healthy volunteers.

IGF-I in vitro study

rhIGF-I was dissolved in RPMI 1640 medium, and 10 μl of different concentrations were added per well. The final IGF-I concentrations per well, assayed by a specific IGF-I assay as described above, were 250, 500, and 1250 μg/liter.

Statistical analysis

In the cross-sectional study statistical comparison between the GH-deficient patients and the age- and sex-matched controls was performed by the Mann-Whitney *U* test. In the IGF-I *in vitro* study comparison between the NK cell activity without and with addition of IGF-I *in vitro* was performed by the Wilcoxon signed rank test. Probability of less than 0.05 was considered significant. All data are shown as the mean ± SE unless otherwise noted.

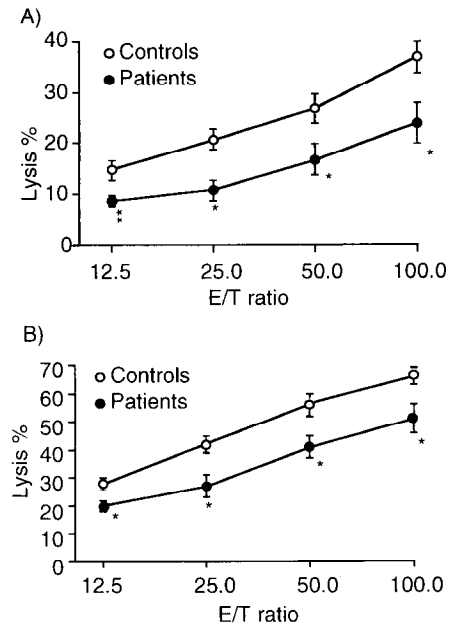

FIG. 1. Basal (A) and IFN-β (B) stimulated NK cell activity in GH-deficient patients and age- and sex-matched controls. A, The basal NK cell activity was significantly reduced in GH-deficient patients in comparison with age- and sex-matched controls at all effector/target cell ratios (E/T). *, $P < 0.05$; **, $P < 0.01$. B, The IFN-β stimulated NK cell activity was significantly reduced in GH-deficient patients in comparison with age- and sex-matched controls at all effector/target cell ratios (E/T). *, $P < 0.05$.

Results

Cross-sectional in vivo study

Total lymphocyte numbers (2.239 ± 0.199 *vs.* 2.020 ± 0.170 g/liter) as well as the percentage and absolute number of $CD3^-/CD16^+$ NK cells (9 ± 2 *vs.* 11 ± 2% and 0.195 ± 0.032 *vs.* 0.222 ± 0.051 G/L) did not differ significantly between the hGH-deficient patients and controls.

The basal as well as the IFN-β stimulated NK cell activity were significantly lower in hGH-deficient patients than in age- and sex-matched controls at all tested effector-target cell ratios from 12.5–100 (Fig. 1). However, stimulation with IFN-β caused an increase of NK cell activity in both groups, and the absolute increase from basal to IFN-β stimulated NK cell activity was not signficantly different in the hGH-deficient patients *vs.* the age- and sex-matched controls.

In the combined analysis of adult hGH-deficient patients and age- and sex-matched controls, Pearsson's correlation performed after assuring normal distribution of data (Kolmogorov-Smirnov test $P > 0.10$) revealed a positive association of IGF-I levels with summarized basal and IFN-β stimulated NK cell activity, which was significant ($R = 0.37$; $P < 0.05$; Fig. 2).

IGF-I in vitro study

In 18 normal subjects IGF-I *in vitro* at final concentrations of 250, 500, and 1250 μg/liter caused a significant increase of basal NK cell activity (14 ± 3% without IGF-I *vs.* 20 ± 3, 23 ± 3, and 22 ± 3% lysis, $P < 0.01$). IFN-β stimulated NK cell activity was also significantly increased by IGF-I *in vitro* (35 ± 4% without IGF-I *vs.* 43 ± 4, 44 ± 5, and 45 ± 5% lysis, $P < 0.001$) (Fig. 3).

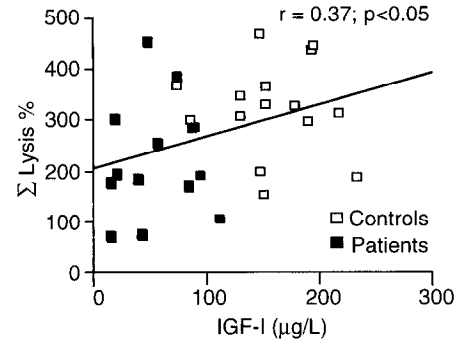

FIG. 2. Association of serum IGF-I levels with the summarized NK cell activity of GH-deficient patients and sex- and age-matched controls.

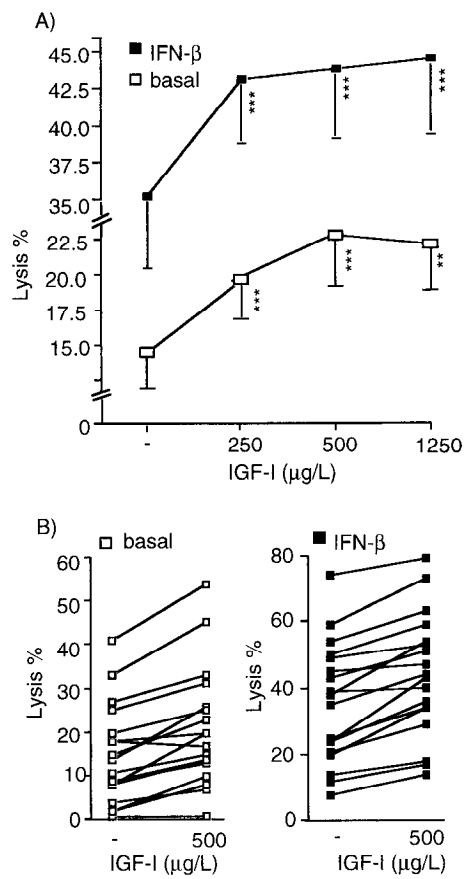

FIG. 3. Effect of IGF-I *in vitro* on basal and IFN-β stimulated NK cell activity in normal subjects. A, The basal and IFN-β stimulated NK cell activity of normal subjects was significantly increased by IGF-I *in vitro* at 250, 500, and 1250 μg/liter. **, $P < 0.01$; ***, $P < 0.001$. B, The increase of the basal and IFN-β stimulated NK cell activity of single normal subjects by IGF-I *in vitro* at 500 μg/liter.

In 13 hGH-deficient adult patients, IGF-I *in vitro* at final concentrations of 250, 500, and 1250 μg/liter also caused a small but significant increase of basal NK cell activity (9 ± 2% without IGF-I *vs.* 10 ± 2, n.s., 10 ± 2, and 11 ± 2% lysis, $P < 0.05$). IFN-β stimulated NK cell activity was also significantly increased by IGF-I *in vitro* (25 ± 4% without IGF-I *vs.* 30 ± 4, 30 ± 4, and 30 ± 4% lysis, $P < 0.01$) (Fig. 4).

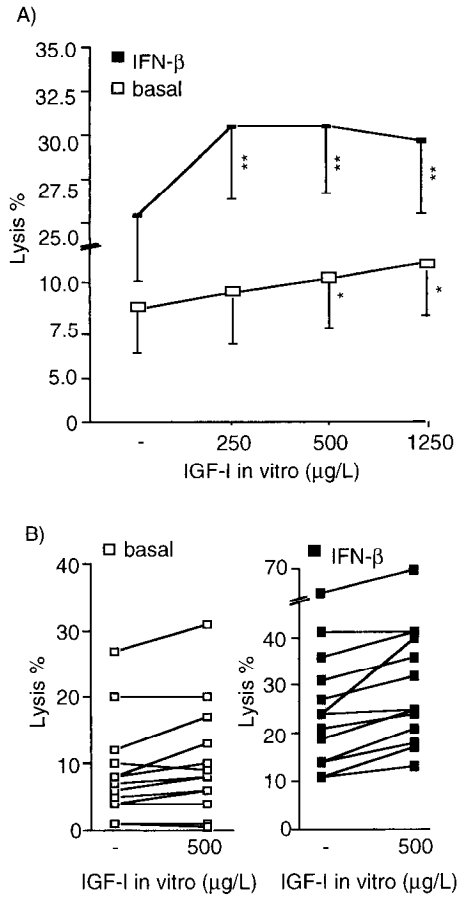

FIG. 4. Effect of IGF-I *in vitro* on basal and IFN-β stimulated NK cell activity in GH-deficient patients. A, The basal and IFN-β stimulated NK cell activity of GH-deficient patients was significantly increased by IGF-I *in vitro* at 250, 500, and 1250 μg/liter. *, $P < 0.05$; **, $P < 0.01$. B) The increase of the basal and IFN-β stimulated NK cell activity of single GH-deficient patients by IGF-I *in vitro* at 500 μg/liter.

Discussion

We have shown that the NK cell activity is significantly decreased in hGH-deficient adult patients in comparison with age- and sex-matched controls despite comparable and normal NK cell numbers. These results are in agreement with several studies on hGH-deficient children describing a decreased NK cell activity despite normal NK cell numbers (11–13). In hGH-deficient children, NK cell activity has been shown to increase during hGH replacement therapy (13). We have also shown that in a combined analysis of hGH-deficient adult patients and age- and sex-matched controls together, the serum IGF-I level correlates significantly with NK cell activity. This analysis was performed after assuring normal distribution of data in both datasets by Kolmogorov-Smirnov test. This finding is in agreement with a study from Crist *et al.* (14), who reported a similar correlation between serum IGF-I levels and NK cell activity in women with impaired endogenous GH secretion. We observed no significant correlation between IGFBP-3 serum levels and NK cell activity (data not shown). However, in adults IGFBP-3 in comparison to IGF-I has been reported to be a rather weak parameter for the assessment of the GH secretory status (22, 23). Therefore, we believe that finding no association between IGFBP-3 and NK cell activity is caused by the poor reflection of the GH-secretory-status by IGFBP-3. To the best of our knowledge there do not exist any data on the *in vitro* effects of GH on NK cell activity or NK number. As IGF-I and GH-receptors have both been demonstrated to exist on NK cells (5–7), in addition to the described effects of IGF-I on NK cell activity, GH may directly play a role in modulating NK cell activity.

In our cross-sectional study of hGH-deficient adult patients the NK cell activity was found to be increased by stimulation with 1000 IU/ml IFN-β *in vitro* to the same extent as in age- and sex-matched controls. These results are in contrast to the findings of Kiess *et al.* (12), who reported NK cell activity of hGH-deficient children not to be stimulated by 1500 IU/ml IFN-α *in vitro* and hypothesized a functional defect of NK cells of hGH-deficient children. These different results might be explained in part by the use of different stimulatory agents and different assay systems in the two studies. We used europium-labeled K-562 cells, which are known to have a shorter lag period of marker release and a higher marker release than ^{51}Cr-labeled K-562 cells (18). In contrast, Kiess *et al.* used ^{75}Se-methionine- or ^{51}Cr-labeled K-562 cells, which show a much lower marker release than the europium-labeled K-562 cells we used. Therfore, Kiess *et al.* might have missed an increase of NK cell activity in hGH-deficient children by IFN-α *in vitro*, because the assay system used was not sensitive enough to measure a small increase of NK cell activity from very low basal values. By contrast, our assay system enabled us to measure NK cell activity over a broad dynamic range of lysis, and we thus may have been able to show an increase of NK cell activity by IFN-β, despite low basal NK cell activity in the hGH-deficient adult patients group. The use of IFN-β instead of IFN-α as used in the study of Kiess *et al.* must also be considered, although both interferons have been described as very potent stimulators of NK cell activity (8). Our findings show an adequate stimulation of NK cell activity of hGH-deficient adult patients and do not indicate a functional defect of NK cells in hGH-deficient adult patients.

Prompted by our observation of a positive association between IGF-I serum levels and NK cell activity, we studied the effect of IGF-I *in vitro* on NK-cell activity. In accordance with the results published by Kooijman *et al.* (6), we demonstrated that basal NK cell activity of normal subjects can be increased by IGF-I *in vitro*. In addition, we also found IFN-β stimulated NK cell activity to be further increased by IGF-I *in vitro*. Using IGF-I *in vitro* in concentrations of 250 μg/liter, 500 μg/liter, and 1250 μg/liter we could not find a clear dose-response relationship between IGF-I and NK cell activity. In serum most of IGFs are bound to IGFBPs (24), whereas in our assay system, by incubating isolated PMNCs in medium containing only 1% human AB serum, a high percentage of "free" IGF-I may have been present. Thus, using probably supraphysiological concentrations of IGF-I in our assay system, we observed almost maximal stimulation of NK cell activity by the lowest concentration of IGF-I tested (250 μg/liter). Further studies therefore should use lower, more physiological concentrations of "free" IGF-I.

IFN-β and IGF-I *in vitro* appear to have an independent,

additive effect on NK cell activity. IFN-β *in vitro* has been reported not to affect the levels of GH mRNA in NK cells, and GH has been found not to induce IFN production (25). To the best of our knowledge, similar data on possible interactions of IGF-I and IFN do not exist.

Furthermore, we were able to demonstrate, that IGF-I *in vitro* can increase the basal and IFN-β stimulated NK cell activity of hGH-deficient adult patients. The increase of basal NK cell activity in hGH-deficient adult patients by IGF-I *in vitro*, although statistically significant, was very small. This may be caused by the low basal NK cell activity of hGH-deficient adult patients being close to the lower detection limit of the NK assay system. At the higher IFN-β stimulated NK cell activities in hGH-deficient patients, however, we could demonstrate a profound increase of NK cell activity by IGF-I *in vitro*. These *in vitro* data and the data on the positive association between serum IGF-I levels and NK cell activity indicate that NK cell activity in hGH-deficient patients might be decreased because of lower serum levels of IGF-I, and NK cell activity can be increased by elevating the IGF-I serum levels. However, an additional direct effect of GH on NK cell activity, teleologically hypothesized because of GH receptor expression on NK cells, still remains to be demonstrated.

In summary, basal and IFN-β stimulated NK cell activity was found to be decreased in hGH-deficient adult patients in comparison to age- and sex-matched controls, despite equal NK cell numbers. NK cell activity was stimulated by IFN-β in hGH-deficient adult patients and in age- and sex-matched controls, indicating that there does not exist a functional defect of the NK cells in hGH-deficient adult patients. Serum IGF-I levels and NK cell activity showed a positive correlation. IGF-I *in vitro* increased basal and IFN-β stimulated NK cell activity in normal subjects and in hGH-deficient adult patients. NK cell activity in GH deficiency might be decreased indirectly, because of lower serum levels of IGF-I. Recently, Watanabe *et al.* (15) suggested GH deficiency to be a risk factor for leukemia and hypothesized low NK cell activity to be a possible explanation for GH deficiency being related to the risk of leukemia. The role of NK cell activity in leukemia is not completely understood (26). In an attempt to elucidate a clinically important aspect of GH deficiency syndrome, further studies should focus on the relationship of GH deficiency, decreased NK cell activity, and the incidence of leukemia.

Acknowledgments

The authors wish to acknowledge valuable discussion and methodological suggestions from Professor B.H. Belohradsky, Dr. von Hauner'sches Kinderspital, Klinikum Innenstadt of the Ludwig-Maximilians-University, Munich; Drs. A. König and C.-D. Pflaum, Medizinische Klinik, for statistical advice; and expert technical assistance from Ms. H. Wuthe and S. Freihöfer.

References

1. **Gala RR** 1991 Prolactin and growth hormone in the regulation of the immune system. Proc Soc Exp Biol Med 198:513–527
2. **Kelley KW, Arkins S, Li YM** 1992 Growth hormone, prolactin, and insulin-like growth factors: new jobs for old players. Brain Behav Immun 6:317–326
3. **Hooghe R, Delhase M, Vergani P, Malur A, Hooghe-Peters EL** 1993 Growth hormone and prolactin are paracrine growth and differentiation factors in the haemopoietic system. Immunol Today 14:212–214
4. **Auernhammer CJ, Strasburger CJ** 1995 Effects of growth hormone and insulin-like growth factor I on the immune system. Eur J Endocrinol 133:635–645
5. **Badolato R, Bond HM, Valerio G, Petrella A, Morrone G, Waters MJ, Venuta S, Tenore A** 1994 Differential expression of surface membrane growth hormone receptor on human peripheral blood lymphocytes detected by dual fluorochrome flow cytometry. J Clin Endocrinol Metab 79:984–990
6. **Kooijman R, Willems M, De Haas CJC, Rijkers GT, Schuurmans AL, Van Buul-Offers SC, Heijnen CJ, Zegers BJ** 1992 Expression of type I insulin-like growth factor receptors on human peripheral blood mononuclear cells. Endocrinology 131:2244–2250
7. **Igarashi N, Takakuwa S** 1994 Flow cytometric analysis of insulin-like growth factor-I (IGF-I) receptors on human blood cells. Clin Pediatr Endocrinol 3(Suppl 5):168–170
8. **Perussia B** 1991 Lymphokine-activated killer cells, natural killer cells and cytokines. Curr Opin Immunol 3:49–55
9. **Lanier LL, Phillips JH** 1992 Natural killer cells. Curr Opin Immunol 4:38–42
10. **Moretta L, Ciccone E, Mingari MC, Biassoni R, Moretta A** 1994 Human natural killer cells: origin, clonality, specificity, and receptors. Adv Immunol 55:341–380
11. **Kiess W, Doerr H, Eisl E, Butenandt O, Belohradsky BH** 1986 Lymphocyte subsets and natural-killer activity in growth hormone deficiency [letter]. N Engl J Med 314:321
12. **Kiess W, Malozowski S, Gelato M, Butenand O, Doerr H, Crisp B, Eisl E, Maluish A, Belohradsky BH** 1988 Lymphocyte subset distribution and natural killer activity in growth hormone deficiency before and during short-term treatment with growth hormone releasing hormone. Clin Immunol Immunopathol 48:85–94
13. **Bozzola M, Cisternino M, Larizza D, Maghnie M, Moretta A, Valtorta A, Schimpff RM, Severi F** 1989 Correspondence: to the editor. Metabolism 38:193
14. **Crist DM, Peake GT, Mackinnon LT, Sibbitt Jr WL, Kraner JC** 1987 Exogenous growth hormone treatment alters body composition and increases natural killer cell activity in women with impaired endogenous growth hormone secretion. Metabolism 36:1115–1117
15. **Watanabe S, Mizuno S, Tsunematsu Y, Komiyama A, Yamanaka C, Kubota M** 1994 Leukemia in GH deficient children. Clin Pediatr Endocrinol 3(Suppl 5):53–60
16. **Boyum A** 1976 Isolation of lymphocytes, granulocytes and macrophages. Scand J Immunol 5(Suppl 5):9–15
17. **Blomberg K, Granberg C, Hemmilä I, Lövgren T** 1986 Europium-labelled target cells in an assay of natural killer cell activity. I. A novel non-radioactive method based on time-resolved fluorescence. J Immunol Methods 86:225–229
18. **Blomberg K, Granberg C, Hemmilä I, Lövgren T** 1986 Europium-labelled target cells in an assay of natural killer cell activity. II. A novel non-radioactive method based on time-resolved fluorescence. Significance and specifity of the method. J Immunol Methods 92:117–123
19. **Maley DT, Simon P** 1990 Cytotoxicity assay using cryopreserved target cells pre-labeled with the fluorescent marker europium. J Immunol Methods 134:61–70
20. **Blum WF, Gallaher B, Ranke MB,** An IGFBP-blocked IGF-I RIA that measures what it pretends to measure: IGF-I. Program of the 74th Annual Meeting of The Endocrine Sociey, San Antonio, TX, 1992, p 968 (Abstract)
21. **Strasburger CJ, Dressendörfer RA, Lee PDK** 1994 Non-isotopic, two-site immunoassay for IGFBP-3. Growth Regul 4(Suppl 1):138 (Abstract)
22. **Cianfarani S, Boemi S, Spagnoli A, Cappa M, Argiro G, Vaccaro F, Manca Bitti ML, Boscherini B** 1995 Is IGF binding protein-3 assessment helpful for the diagnosis of GH deficiency? Clin Endocrinol (Oxf) 43:43–47
23. **de Herder WW, van der Lely AJ, Janssen JAMJL, Uitterlinden P, Hofland LJ, Lamberts SWJ** 1995 IGFBP-3 is a poor parameter for assessment of clinical activity in acromegaly. Clin Endocrinol (Oxf) 43:501–505
24. **FrystykJ, Skjaerbaek C, Dinessen B, Orskov H** 1994 Free insulin-like growth factors (IGF-I and IGF-II) in human serum. FEBS Lett 348:185–191
25. **Weigent DA, Blalock JE** 1991 The production of growth hormone by subpopulations of rat mononuclear leukocytes. Cell Immunol 135:55–65
26. **Pross HF, Lotzova E** 1993 Role of natural killer cells in cancer. Nat Immun 12:279–292