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

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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 vitro 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 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 positive correlation with NK cell activity. In an in vitro study, IGF-I significantly enhanced basal and IFN-β stimulated NK cell activity of GH-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 Kiese 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 vitro 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 arginine 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 adenoma (n = 4), prolactinoma (n = 2), craniopharyngioma (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), T4 (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 ± SD), 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...
levels of GH (0.3 ± 0.1 vs. 2.4 ± 0.9 pg/liter; P < 0.05), IGF-I (60 ± 12
vs. 159 ± 12 pg/liter; P < 0.001), and IGFBP-3 (2175 ± 306 vs. 3558 ±
184 pg/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.

In the IGF-I in vitro study, NK cell activity of 13 hGH-deficient pa-
tients (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, Paisley, Scotland. Human AB serum, HEPES, DMSCO, 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, MgCl2, CaCl2, glucose, and DTPA were purchased from
Merck, Darmstadt, Germany. Dextrane sulphate was from Pharmacia,
Uppsala, Sweden. Murine anti CD3 and anti CD16 monoclonal anti-
bodies were from Dianova, Hamburg, Germany. The lysis and fixation
solution for flow cytometry were from Coulter, Krefeld, Germany. Eu-
ropium-labeled and cryopreserved 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 glu-
tamic acid, penicillin/streptomycin, and amphotericin B. Cells were ad-
justed to 2 X 10^6 cells/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 plates at 1 X 10^5, 5 X 10^5, 2.5 X 10^5, 10 X 10^5,
and 100 X 10^5 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 re-
olved 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 mea-
surement 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
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 200–1500 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
cell were measured 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.
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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 significantly 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, Pearson’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, 22 ± 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).

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).
have also shown that in a combined analysis of hGH-deficient children, NK cell activity has been shown to increase during hGH replacement therapy (13). We increased NK cell activity despite normal NK cell numbers in several studies on hGH-deficient children describing a decreased 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 51Cr-labeled K-562 cells (18). In contrast, Kiess et al. used 75Se-methionine- or 51Cr-labeled K-562 cells, which show a much lower marker release than the europium-labeled K-562 cells we used. Therefore, 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. 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.

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