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Antiproliferative Effect of Hu-Interferon-Gamma in 674V and J82 Bladder Carcinoma Cell Lines

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Summary. Hu-IFN-gamma was evaluated in regard to the antiproliferative effect on J82 and 647V bladder cancer cell lines. In addition, the IFN-receptors were determined. There was a significant growth inhibition of J82 as well as 647V at low dose Hu-IFN-g (1 U/ml). The growth inhibition was significantly higher in 647V than in J82. The binding assay for 125J-Hu-IFN-g revealed 870 and 3,000 binding sites for 647V and J82, respectively, indicating that the antiproliferative effect of Hu-IFN-g may not depend on the absolute amount of IFN-receptors, in the two cell lines tested.

Key words: Interferon-gamma – Receptors – Bladder cancer

Introduction

Human interferon-gamma (hu-IFN-g) mediates immune function and has distinct effects as an antiproliferative and as an antiviral agent [2, 5, 6]. Talmadge et al. reported on an increased efficacy of hu-IFN-g compared to other interferons [14]. Moreover these authors observed an increasing growth reduction with higher dosages. Animal experiments revealed similiar results [12].

There are specific receptor sites for hu-IFN-g, which differ in density not only within tumors of different origin but also in the same histological tumor type as well [11, 13, 15]. Moreover it remains to be shown, whether there is a correlation between the receptor density and the antiproliferative as well as immune modulatory effects [13, 15].

Since IFN-alpha has a limited value in human bladder cancer, we evaluated hu-IFN-g in regard to antiproliferative activity as well as receptor sites to show whether hu-IFN-g should be used in clinical studies.

Materials and Methods

Reagents

Hu-IFN-g from Genentech, San Francisco, CA, was provided by G. Adolf E. Boehringer Institute, Vienna, Austria. Hu-IFN-g was derived from recombinant DNA, and the purity was greater than 99.9%. The specific activity was 3.2×10^7 U/mg protein. The IFN-solutions in phosphate-buffered saline were diluted in Dulbecos medium supplemented with 15% FBS and stored at −70 °C.

Cell Culture

The human bladder carcinoma cell lines J82 and 647V were purchased from American Cell Type, Rockville, USA. The cell lines were cultured in Dulbeco's medium supplemented by 15% FBS, 2 mM glutamine, non essential amino acids (1%), penicillin and streptomycin. For stock culture, cells were grown as monolayer in T75 plastic flasks (Nunc, Roskilde, Denmark) in a humified atmosphere (5% CO_2 – 95% air) at 37 °C. For the determination of growth effects, cells from stock flasks were harvested and then seeded in Nunc 24-well tissue culture plates as 1-ml suspensions in Dulbecco's supplemented with 15% FBS as previously described [10]. The cells were allowed to attach for 24 h, and then the medium was changed to one containing the appropriate concentration of IFN; the medium was renewed every 2–3 days. For the construction of growth curves, the cell number was determined by means of a coulter counter at the beginning of each experiment and at predeterm ined intervals. Viability of counted cells was routinely checked for representative specimens by trypan blue exclusion and was always greater than 90%.

Binding Assay

Radioiodination of Hu-IFN-g was performed by the method of Bolton and Hunter [3] with further modification by Anderson [1]. In brief, 2×10^7 U/mg Hu-IFN-g were added to 1 mCi 125 J-Bolton Hunter reagent (NEN) at 4 °C. After 1 h the reaction was stopped by the addition of glycine. The iodinated sample was applied to a Sephadex G-25 (fine) column. The specific activity of this preparation was 581 Ci/mmol. Fractions containing the iodinated protein were pooled.

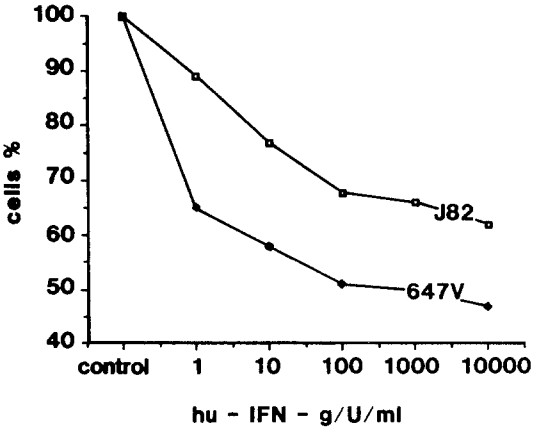

Fig. 1. Growth inhibition in percentage of control cells with increasing dosage of IFN-g

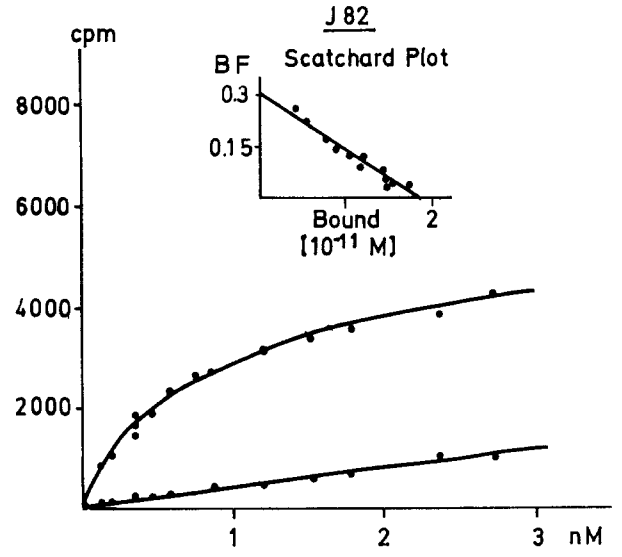

Fig. 2. Scatchard plot of specific IFN-g binding to 647V bladder cancer cell line

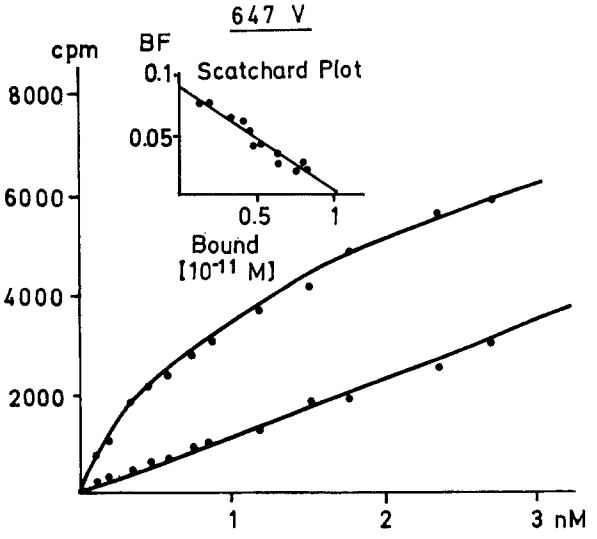

Fig. 3. Scatchard plot of specific IFN-g binding to J82 bladder cancer cell line

The binding assays were performed as previously described by Ücer et al. [15]. Cells were carefully washed twice with TMC buffer, pH 7.4 containing 0.9 NaCl and 1% BSA and finally resuspended (647V –11.75 x 106/ml; J82 –6.17 x 106/ml). Aliquots of the suspension (0.3 ml) were incubated with an increasing concentration (0.15 nM–2.67 nM) of 125 J-Hu-IFN-g alone or in the presence of a 500 fold excess of unlabeled Hu-IFN-g. After incubation for 4 h at 0 °C the samples were centrifuged and the supernatants were removed by aspiration. The pellets were washed twice with 1 ml incubation buffer. Radioactivity was determined in a gamma-counter.

Statistical Evaluation

Growth curves were analyzed by the Wilcoxon matched pairs signed rank test or by the two-way analysis of variance by ranks according to Wilcoxon and Wilcox.Multiple comparisons were performed by the Kruskal-Wallis one-way analysis of variance. These results were also checked by the U-test according to Wilcoxon-Mann-Whitney.

Results

There was a significant antiproliferative effect of Hu-IFN-g in both cell lines. This effect was more pronounced for 647V than J82 cells (Fig. 1). The antiproliferative effect was already seen at low dose and could not be increased by dosages higher than 100 U/ml Hu-IFN-g.

On the surface of both bladder carcinoma cell lines highly specific binding sites for 125 J-hu-IFN-g were detected (Figs. 2, 3). The number of receptor molecules per cell was higher for J-82 with 3,000 than for 647-V with 870 binding sites per cell ($P < 0.05$). The affinity of the receptor for its ligand was statistically not distinguishable between the two cell lines and the KD ranged from 60 to 110 pM. The binding obtained with each cell line was highly reproducable when assayed in repeated experiments.

Discussion

Growth inhibition of various tumor cell lines were observed by partially purified preparations of murine and human IFN-gamma [2, 5]. Le et al. demonstrated that recombinant Hu-IFN-g has significant cytostatic activity in colon carcinoma and rhabdomyosarcoma cell line [8]. Thus indicating that the effects seen by the previous authors were not due to impurity of the IFN-g used. The mode of the antiproliferative activity of Hu-IFN-g still remains to be established [13, 15]. It was shown by Lee et al. that incubation of tumor cell lines W138 and W138VA13 with Hu-IFN-g induces an accumulation of tumor cells in the Go/G1 phase of the cell cycle [9]. Furthermore, Bruchelt et al. demonstrated that [2–5] A-oligo-isoadenylate-synthetase (OAS) is significantly increased in solid tumor cell lines incubated with hu-IFN-g [4]. But they also showed that high OAS levels are not necessarily connected with cell growth inhibition. The sensitivity of tumor cell lines to Hu-IFN-g varies significantly [5, 6, 13]. This can be demonstrated not only in vitro but also in animal experiments [2].

It is a well established fact that Hu-IFN-g binds to a specific receptor, which is quite distinct from that which binds to IFN-alpha and IFN-beta [7, 13]. It was speculated that the ineffectiveness of IFN-g may be related to a low density of cell surface receptors. Nagao et al. and Ücer et al. correlated the antiproliferative effect of Hu-IFN-g and receptor density with a panel of tumor cell lines [11, 15]. They did not find any consistency between receptor density and antiproliferative effect. Moreover, it is evident, that there are tumor cells which are complete resistant to Hu-IFN-g and that this does not correspond with a lack of binding of IFN-g [13].

Both tumor cell lines investigated in this study showed a high density of receptor sites as well as a high affinity to the binding sites. However, the antiproliferative effect was the highest in the cell line with the lower density of receptor sites.

Since we know little about the molecular interactions of IFN bound to the receptor, further investigation should be directed to this aspect. Moreover, our interest is focused on the synergism of IFN-g with cytostatic drugs as well as other biological response modifiers [8, 9]. Since an antiproliferative effect against bladder cancer cell lines was shown hu-iFN-g may by used in controlled clinical trials similiar to those which were performed with IFN-alpha.

References

1. Anderson P, Yum KY, Vilcek J (1983) Human IFN-gamma is internalized and degraded by cultured fibroblasts. J Biol Chem 258:6497–6502
2. Balkwill FR, Goldstein L, Stebbing N (1985) Differential action of six human interferons against two human carcinomas growing in nude mice. Int J Cancer 35:13–17
3. Bolton AE, Hunter WM (1973) The labelling of proteins to high specific radioactivities by conjugation to a 125 J-containing acylating agent. Biochem J 133:529–539
4. Bruchelt, Treuner J, Beck J, Schilling F, Koscielniak E, Niethammer D (1985) IFN-gamma potentiates the induction of (2–5) A-synthetase in human tumor cells treated with IFN-alpha and beta. In: The 2–5A system: molecular and clinical aspects of the interferon-regulated pathway. Liss, New York, pp 391–395
5. Denz H, Lechleitner M, Marth C, Daxenbichler G, Gastl G, Braunsteiner H (1985) Effect of human recombinant alpha-2- and gamma-interferon on the growth of human cell lines from solid tumors and hematological malignancies. J Interferon Res 5:147–157
6. Gastl G, Marth C, Leiter E, Gattringer C, Mayer I, Daxenbichler G, Flener R, Huber C (1985) Effects of human recombinant alpha 2 arg-interferon and gamma-interferon on human breast cancer cell lines: dissociation of antiproliferative activity and induction of HLA-DR antigen expression. Cancer Res 45:2957–2961
7. Hannigan GE, Fish EN, Williams BRG (1984) Modulation of human interferon-alpha receptor expression by human interferon-gamma. J Biol Chem 259:8084–8086
8. Le J, Yip YK, Vilcek J (1984) Cytolytic activity of interferon-gamma and its synergism with 5-fluorouracil. Int J Cancer 34:495–500
9. Lee SH, Aggarwal BB, Rinderknecht E, Assisi F, Chiu H (1984) The synergistic anti-proliferative effect of gamma-interferon and human lymphotoxin. J Immunol 133:1083–1086
10. Marth C, Daxenbichler G, Buehring GC, Hofstädter F, Dapunt O (1984) Inhibition of estradiol-induced growth of cultured human breast cancer cells by the anti-estrogens tamoxifen, desmethyl-tamoxifen, 4-hydroxy-tamoxifen and enclomiphene. Biochem Pharmacol 33:3951–3956
11. Nagao S, Sato K, Osada Y (1986) Binding of human-interferon to human epidermal tumor cells with different susceptibilities. Cancer Res 46:3279–3282
12. Otto U, Huland H, Zschaber R (1985) Recombinant interferon gamma: first results of a clinical phase II study in patients with metastastic renal cell carcinoma and experimental studies of renal cell carcinoma transplantated into nude mice (Abstract 168). J Urol 133:155A
13. Pfitzenmaier K, Bartsch H, Scheurich P, Seliger B, Ucer U, Vehmeyer K, Nagel GA (1985) Differential gamma-interferon response of human colon carcinoma cells: inhibition of proliferation and modulation of immunogenicity as independent effects of gamma-interferon on tumor cell growth. Cancer Res 45:3503–3509
14. Talmadge JE, Tribble HR, Pennington RW, Phillips H, Wiltrout RH (1987) Immunomodulatory and immunotherapeutic properties of recombinant gamma interferon and recombinant tumor necrosis factor in mice. Cancer Res 47:2563
15. Ucer U, Bartsch H, Scheurich P, Pfitzenmaier K (1985) Biological effects of gamma-interferon on human tumor cells: quantity and affinity of cell membrane receptors for gamma-IFN in relation to growth inhibition and induction of HLA-DR expression. Int J Cancer 36:103–108

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