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BIOLOGICAL AND CLINICAL RESPONSE OF RECOMBINANT INTERFERON GAMMA IN PATIENTS WITH ADVANCED RENAL CELL CANCER

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INTRODUCTION:

Interferon (IFN) gamma is a potent immunomodulatory agent with a wide range of biological properties (1). It enhances the functional activity of various immune effector cells. In addition, IFN gamma regulates the interaction of MHC restricted cytotoxic T cells with nonhemopoetic cells by inducing the synthesis of HLA products (2). Thus this compound might be an excellent candidate for modifying the hosts' immune response to malignant disease.

There is some evidence suggesting that immune mechanisms are critical for the control of renal cell cancer. First, spontaneous remissions are infrequently seen after tumor nephrectomy in patients with metastasizing RCC (3). Second, in almost all studies using biological response modifiers for treatment of advanced renal cell cancer tumor regressions were observed in some patients (4,5,6,13).

Most studies using biological response modifiers for treatment of advanced cancer applied the maximum tolerated dose assuming that this dose should be the optimum antiproliferative dose.

TABLE 1

n	Age	Sex	Karnovsky	TNM	Metastases	Response
1	75	m	70	T3N1M1	liver, local	MR
2	57	m	60	T4N2M1	liver, lung	PRO
3	66	m	100	T3NXM1	lung	SD
4	55	m	80	T4N4M1	lymph node,lung	PR
5	67	m	100	T4N1M1	lung	n.e.*)
6	68	m	90	T2NOM1	lung	SD
7	66	m	90	T4NOM1	lung	CR
8	57	m	90	T3N2M1	lung	PRO
9	57	m	100	T3NXM1	liver	PRO
10	50	f	90	T3NOM1	lung, CNS	PRO
11	69	m	70	T4NXM1	lung,lymph node	PRO
12	46	m	90	T4NXM1	lung	CR
13	41	m	70	T2NXM1	bone	n.e.*)
14	62	m	90	T3NOM1	lung,liver	PRO
15	54	m	90	T3NOM1	lung,bone	PRO
16	68	f	100	T3N2M1	lung	PR

Table 1. Patients characteristics and clinical response after 12 months.

However, there is increasing evidence from experimental animal studies and clinical trials that the maximum tolerable dose does not represent the optimum immunomodulatory dose (7,8,9). Thus, if immunomodulation is one of the critical mechanisms of action for antitumor activity, new strategies are required for identification and definition of biologically and clinically effective doses of cytokines for cancer treatment.

MATERIAL AND METHODS:

The study reported was performed according to the Declaration of Helsinki. The protocols were approved by the local Ethics committee. All patients had given their written informed consent prior to start of treatment.

16 patients suffering from progressive metastasizing renal cell carcinoma were treated with 10, 100 and 500 µg recombinant interferon gamma. Fourteen of the patients were male and 2 female. Their ages ranged from 41 to 75 years (median 59). Major manifestation of the disease were lung, bone and liver metastases. The clinical characteristics of the patients are summarized in table 1 (Pat 1-16).
Recombinant IFN gamma, originally produced by Genentech Inc., with a specific activity of 2x10 E7 I.U./mg protein was obtained from Boehringer Ingelheim International (Ingelheim, FRG). Some of the data were reported in detail previously (13,14,15).

TREATMENT PROTOCOL

Treatment consisted of two consecutive phases. During an initial dose finding phase three different doses of r-IFN gamma (10, 100, 500 µg)were administered subcutaneously (s.c.). Each dose was administered 3-times to the same individual. The sequence of the dose levels was randomly assigned. In order to prevent carry over effects a therapy -free interval of 2 weeks was introduced between each dose level.
Blood was drawn 1 hour before and 4, 12, 24, 48, 72, 96, 120 and 167 hours after the IFN-gamma injections.

EVALUATION OF TUMOR RESPONSE AND DRUG-INDUCED SIDE EFFECTS:

Tumor response and severity of side effects were defined according to WHO criteria (10). Quantitative changes of tumor size were assessed at least every four weeks by clinical examination, abdominal sonography, chest x-ray studies and CT scanning. All patients were assessed by more than one method of evaluation.

LABORATORY INVESTIGATIONS:

Differential blood counts were performed using established standard methods. Briefly, cell counts were assessed with a coulter counter and differential blood counts were performed on Giemsa stained blood smears by counting a minimum of 200 cells.

Numbers of leukocyte subsets in the periphery were determined by staining cytocentrifuge preparations of Ficoll separated peripheral blood mononuclear cells with an indirect immune-peroxidase method using Leu3, Leu2a, Leu1, Leu7.
Anti HLA DR (Beckton Dickinson Inc., USA) as primary antibodies.

Serum levels of TNF alpha were determined by commercially available immunoradiometric assays (IRMA) (Medgenix Brusseles, Belgium). The lower limit of detection using these tests is > 5 pg/ml for TNF alpha and > 0.2 U/ml (NIH standard) for IFN gamma. Neopterin serum levels were determined by a commercially available radioimmunoassay (Henning Inc., Berlin FRG).

STATISTICS:

Statistical significance of the differences before and after

application of the cytokines were analyzed by means of the confidence limits of the mean difference (13) or by Wilcoxon matched-pairs signed-rank test and Mann-Whitney's U test using a commercially available statistical software package (SPSS Inc, Chicago, USA).

RESULTS:

CLINICAL RESPONSE:

Two of the 16 patients enrolled did not complete the dose finding phase and were therefore excluded from response evaluation: patient no. 5 experienced rapid disease progression during the first three weeks of therapy and patient no. 13 refused further treatment after six weeks due to severe constitutional symptoms following administration of 500 µg of IFN-gamma.
14 patients completed the dose finding phase and entered the phase II efficacy trial receiving r-IFN-gamma 100 µg s.c. once weekly. During a median time of treatment of ten months (range, 2-32 months) complete or partial responses were observed in 4 of 14 evaluable patients. In all 4 responses were seen within three months of initiating IFN-gamma. All 4 complete and partial responses were seen in patients with lung metastases (table 1).

SIDE EFFECTS:

During the dose-finding phase fever, fatigue and chills were the most frequent side effects. Both frequency and severity of these symptoms were clearly dose-dependent. Grade 2 toxicity (WHO-grading) was observed in twelve of 14 cases during treatment

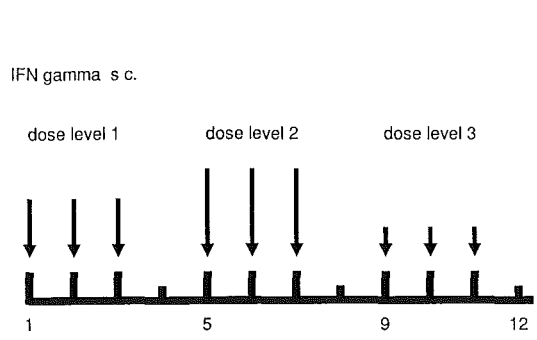

Figure 1. Study design

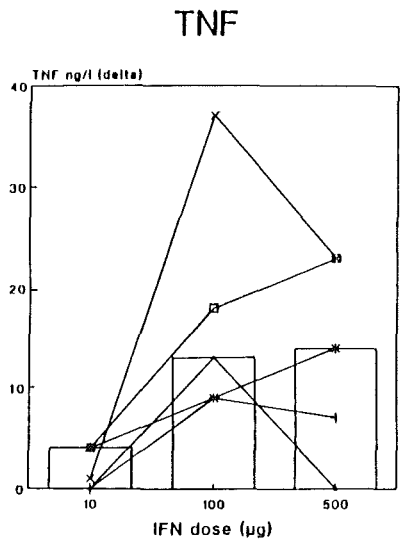

Figure 2. Dose depending of the increase of TNF release after treatment with IFN-γ. Delta values are calculated from pretreatment and peak values. Bars represent median values.

with 500 µg of IFN-gamma. This was seen in half of the patients receiving 100 µg of IFN-gamma and in only three patients at the 10 µg dose level.

Two patients refused further treatment due to side effects after 500 µg r-IFN-gamma.

TUMOR NECROSIS FACTOR ALPHA SERUM LEVELS AFTER TREATMENT WITH RECOMBINANT INTERFERON GAMMA:

TNF alpha serum levels were measured in 282 serum samples of 5 patients. Only two out of five patients had detectable serum levels of TNF alpha in pretreatment samples. Administration of recombinant IFN gamma led to a statistically significant increase of TNF alpha serum levels in almost all cases (p 0.01) (Fig. 2).

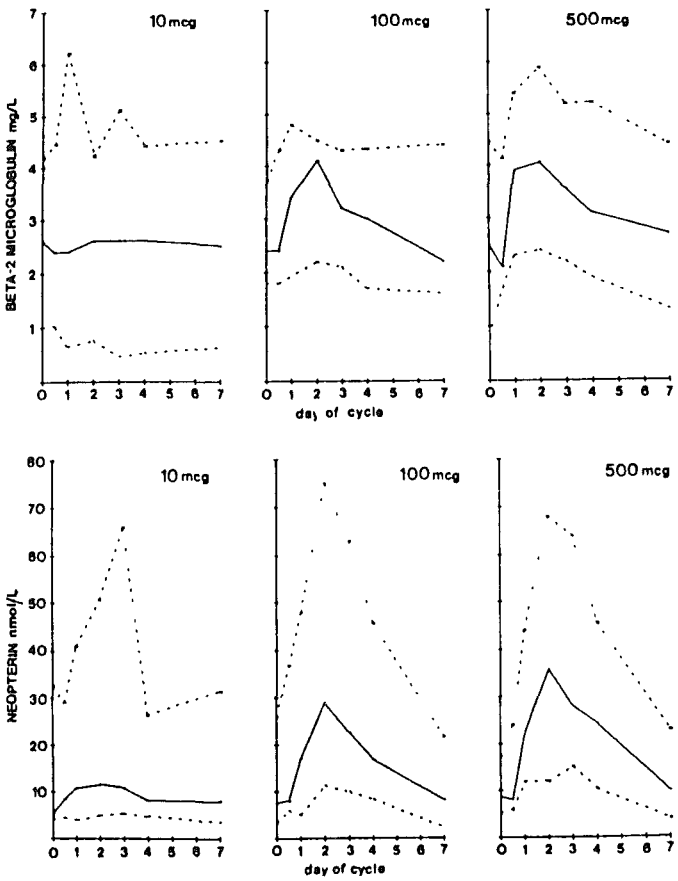

Figure 3. Neioterin and Beta-2-Microglobulin release after single injections of IFN-gamma in 16 patients with m-RCC (median values and range).

TNF levels peaked 24 hours after application of r-IFN gamma and returned to baseline values within four days.

NEOPTERIN AND BETA-2-MICROGLOBULIN AFTER TREATMENT WITH r-IFN GAMMA:

Neopterin is a metabolic product of monocytes activated by IFN gamma (11). Neopterin levels increased in all patients after

TABLE 2

IFN-γ dose	10 µg	100 µg	500 µg
Leukocytes			
4 h	101% (74-129)	82% (64-118)[a]	98% (46-123)
24 h	88% (55-113)	83% (45-149)[b]	73% (32-97)[b]
Granulocytes			
4 h	102% (66-165)	99% (66-146)	97% (48-201)[c]
24 h	101% (60-140)	90% (41-187)[a]	76% (30-131)[b,c]
Lymphocytes			
4 h	96% (36-195)	85% (54-145)	69% (29-196)[b]
24 h	75% (43-137)[a]	66% (23-114)[b]	65% (39-145)[b,d]
Monocytes			
4 h	90% (7-405)	57% (0-215)[a]	35% (0-400)[b,e]
24 h	127% (0-392)	66% (0-459)	54% (0-357)[b]

[a] $p < 0.05$ when compared to pretreatment values.

[b] $p < 0.01$ when compared to pretreatment values.

[c] $p < 0.01$ when response to 10 µg and to 500 µg are compared.

[d] Not significant when response to 10 µg and to 500 µg are compared.

[e] $p = 0.055$ when response to 10 µg and to 500 µg are compared.

Table 2. Response of white blood cell counts to single doses of IFN-γ expressed as a percentage of pretreatment values.

100 µg and 500 µg of IFN gamma peaking at 48 hours after the application (Fig 3). As shown in the figure, nearly maximum

TABLE 3

IFN-γ dose	10 µg	100 µg	500 µg
Leu1 T lymphocytes			
4 h	109% (50-177)	116% (43-173)[a]	84% (30-167)[a]
24 h	88% (46-151)	66% (14-151)	79% (23-157)[b]
Leu3 T lymphocytes			
4 h	132% (50-238)	133% (53-215)	90% (40-283)
24 h	86% (46-177)	75% (20-154)	82% (26-154)
Leu2a T lymphocytes			
4 h	88% (38-180)	122% (60-159)	76% (25-114)[c,a]
24 h	74% (32-185)	61% (13-183)[c]	63% (23-193)[c,a]
Leu7* NK cells			
4 h	82% (32-220)[c]	63% (24-197)[c]	38% (0-139)[c,a]
24 h	69% (18-414)[c]	39% (8-351)[b]	32% (6-130)[b,a]
HLA DR* cells			
4 h	70% (20-212)	51% (21-245)[c]	38% (25-127)[b,a]
24 h	88% (56-132)	85% (35-249)	70% (39-176)[c]
T4/T8 ratio			
4 h	130% (44-271)	118% (76-205)	126% (66-217)[c]
24 h	121% (46-234)	110% (77-264)	122% (56-326)[b]

[a] $p < 0.05$ when response to 10 µg and 500 µg are compared.

[b] $p < 0.01$ when compared to pretreatment values.

[c] $p < 0.05$ when compared to pretreatment values.

Table 3. Response of T-lymphocytes subsets to single doses of r-IFN-γ expressed as a percentage of pretreatment values.

induction was achieved with 100 µg r-IFN-gamma and increasing the dose to 500 µg r-IFN-gamma caused only a marginally higher induction of the synthesis of this molecule. An identical pattern was observed with beta-2-microglobulin after treatment with single doses of r-IFN-gamma (16). Significant induction was observed already after treatment with 100 µg r-IFN gamma. Administration of 500 µg r-IFN-gamma was followed by a slightly higher peek.

ACUTE EFFECTS ON THE COMPOSITION OF PERIPHERAL BLOOD LEUCOCYTES:

IFN-gamma treatment caused only minor effects on total white blood cell counts. However the relative composition of various leucocyte subsets changed markedly upon treatment with IFN-gamma. Granulocyte counts were not effected, whereas the numbers of lymphocytes and monocytes decreased after administration of cytokines reaching the nadir two to twentyfour hours after injection.

The highest dose of IFN-gamma lowered circulating monocytes by approximately 70 % and circulating lymphocytes marginally ($\sim$ 30 %). Numbers returned to pretreatment values within 48 hours.

T-CELL SUBSETS:

Lymphocyte subsets after IFN-γ administration showed only minor changes. The number of circulation Leu 1* cells declined 24 hours after 500 µg IFN-gamma from a median value of 629 cells/µl to 608 ($p < 0.01$) (Table 3). The reduction after 10 µg and 100 µg did not reach the level of statistical significance (Table 2). Leu3* cells increased slightly 4 h after application of the two lower dose levels, whereas 500 µg resulted in decreasing cell counts. In contrast, the number of Leu2a* cells in the peripheral

blood fell significantly with a nadir 24 h after administration of both 100 µg and 500 µg of IFN-γ (from a median of 220 cells/µl to 120 cells/µl, $p < 0.05$). The divergent behavior of Leu3* and Leu2a* cells resulted in elevated T4/T8 ratios.

DISCUSSION:

Three major conclusions can be derived from the results of this study: First, significant modification of biological response was observed at dose levels far below the maximum tolerated dose. r-IFN gamma effects in this low dose range were demonstrated on homing properties of peripheral blood leucocytes, synthesis of HLA proteins and neopterin, and activation of the cytokine network. These findings are in conformity with results of other groups, were an optimum immunomodulatory dose of 100 µg was proposed (9). Second treatment of patients with advanced renal cell cancer with low dose of r-IFN gamma can induce tumor regressions in these patients. Moreover, the remission rate observed in our study was within the range as those observed after treatment of RCC patients with IFN alpha, IL-2, IL-2 plus IFN alpha, IL-2 + LAK cells (4,5,6). However, patients treated with low dose gamma interferon experienced considerably less toxicity than reported from studies applying tolerable doses of cytokines. Previous reports of r-IFN gamma for treatment of advanced RCC demonstrated an extremely variable antitumor activity in RCC patients with response rates ranging from 0 to 30 % (12). It cannot be excluded that differences in patient selection account for varying clinical results. Nevertheless, the fact that responses were seen almost exclusively in patients treated intermittently with r-IFN gamma might indirectly support the view, that continuous intravenous treatment with maximum

tolerable doses is not the appropriate way for using r-IFN gamma in these patients. We therefore conclude that the low dose range of r-IFN gamma has to be further explored in these patients.

The last important observation of this study is that biological response seems to be a prerequisite for tumor response in RCC patients. All patients who showed no signigicant increase of beta-2-microglobulin levels after treatment with r-IFN gamma were resistant to therapy (13). Thus these marker molecules might be appropriate parameters for the action of r-IFN gamma and be thereby a means of designing optimal immunomodulatory treatment schedules.

REFERENCES

1. Gastl G, Huber C (1988). The Biology of Interferon Action. Blut 56:193-199.

2. Niederwieser D., Auböck J., Troppmair J., Beck P., Fritsch P., Huber Ch. (1988). IFN mediated induction of MHC expression on human keratinocytes and its influence on in vitro immune response. J Immunol, 140:2556-2564

3. Oliver RTD, Miller RM, Mehta A, Barnett MJ. A Phase II study of surveillance in patients with metastatic renal cell carcinoma and assessment of response of such patients to therapy on progression. Mol Biother 1988 1:14-20

4. Rosenberg SA, Lotze MT, Muul LM et al (1988). A progress report on the treatment of 157 patients with advanced cancer using lymphokine activated killer cells and interleukin 2 or high dose interleukin 2 alone. N Eng J Med 316:889-897

5. Neidhart JA (1986) Interferon therapy for the treatment of renal cell cancer. Cancer 57:1696-1699

6. Quesada JR. (1988) Biological response modifiers in the therapy of metastatic renal cell carcinoma. Seminars in Oncology 15:396-407

7. Talmadge JE, Tribble HR, Pennington RW, Philipps H, Wiltrout RH. (1987): Immunomodulatory and immunotherapeutic properties of recombinant gamma interferon and recombinant tumor recrosis factor in mice. Cancer Res, 47:2563-2570

8. Kleinerman ES, Kurzrock R., Wyatt D, et al. Activation or suppression of the tumoricidal properties of monocytes from cancer patients following treatment with human recombinant interferon gamma. Cancer Res 46:5401-5405

9. Maluish A., Urba W.J., Longo D.L. et al. (1988). The determination of an immunologically active dose of interferon gamma in patients with melanoma. J Clin Onc 6:434-445

10. WHO handbook for reporting results of cancer treatment. WHO, Geneva, 1979

11. Huber C, Batchelor SR, Fuchs D, Hausen A, Lang A, Niederwieser D, Reibnegger G, Swetly P, Troppmair J, Wachter H (1984): Immune response associated production of neopterin. Release from macrophages primarily under control of IFN gamma. J Exp Med, 160:310-316

12. Aulitzky W.E., Aulitzky W., Lttichau I., Huber CH. Treatment of advanced renal cell carcinoma with recombinant Interferon gamma. Interferons and cytokines (in press).

13. Aulitzky W., Gastl G., Aulitzky W.E., Herold M., Kemmler J., Mull B., Frick J. and Huber C. (1989). Successfull treatment of metastatic renal cell carcinoma with a biologically active dose of recombinant interferon gamma. J Clin Oncol,7, 1875-1884.

14. Aulitzky W.E., Aulitzky W., Gastl G., Lanske B., Reitter J., Frick J., Tilg H., Berger M., Herold M. and Huber C. (1989). Acute effects of single doses of recombinant IFN gamma on blood cell counts and lymphocyte subsets in patients with advanced renal cell cancer. J.Interferon Res. 9, 425-433.

15. Aulitzky W.E., Aulitzky W., Frick J., Herold M., Gastl G., Tilg H., Berger M. and Huber C. (1988). Treatment of cancer patients with recombinant interferon gamma induces release of endogenous tumor necrosis factor alpha. Immunobiology 1990 180:385-394

16. Cytokines in the control of beta-2-microglobulin release 2. In vivo studies with recombinant interferons and antigens K. Nachbaur, J. Troppmair, B. Kotlan, P. König, W. Aulitzky, P. Bieling, Ch. Huber Immunbiol. 1988, Vol. 177, S 66-75

Discussion -BIOLOGICAL AND CLINICAL RESPONSE OF RECOMBINANT INTERFERON GAMMA IN PATIENTS WITH ADVANCED RENAL CELL CANCER

M.M. Reidenberg

I think this was a very important paper. The traditional cytotoxic drugs used in cancer have an ordinary dose response relationship and it was really Dr. Skipper popularizing the fractional kill hypothesis that lead to the present conventional treatment of cancer using the highest tolerated dose of multiple cytotoxic drugs. When cytokines were then introduced into therapeutics the same concept of really pushing the dose to toxicity and then backing off a little bit was used. And the results were disappointing. Now, Dr. Aulitzky is presenting really good clinical pharmacological data suggesting that the conceptual basis of treating cancer based on the normal dose response of cytotoxic drugs and the Skipper Hypothesis just isn't appropriate when we are dealing with biological response modifiers.

L. Gauci

I remember the arguments that occurred in two of the major pharmaceutical companies that were involved firstly with IFN alpha and then with IFN gamma development. There was one opinion which said there was only one way to develop a cancer drug and that is to give it at high doses like chemotherapy, but there was a small group of timorous people who said that these were biologicals and perhaps we ought to target them differently. The development of alpha IFN was rescued because of a rare selective indication, hairy cell leukemia, and gamma IFN was saved because of congenital granulomatosis.

W. Aulitzky

I think, that we have to make an important step in clinical research. We have to go back into man as a big test tube. We have to do it very carefully and we must not do any harm. we also have to go back into man because that is the only environment where we can study all those effects and interactions we need to study. We can't do it in simple in vitro systems.