Alhava E. Clinical evaluation of a new serum tumour marker CA-242 in pancreatic carcinoma. *Br J Cancer* 1992, 65, 731–734.
16. Haglund C, Lindgren J, Roberts PJ, Kuusela P, Nordling S. Tissue expression of the tumour associated antigen CA-242 in benign and malignant pancreatic lesions. *Br J Cancer* 1989, **60**, 845–851.
17. Murata K, Egami H, Shibata Y, Sakamoto K, Misumi A, Ogawa M. Expression of blood group-related antigens, ABH, Lewis^a, Lewis^b, Lewis^x, Lewis^y, CA19-9, and CSLEX1 in early cancer, intestinal metaplasia, and uninvolved mucosa of the stomach. *Am J Clin Pathol* 1992, **98**, 67–75.

Eur J Cancer, Vol. 29A, No. 14, pp. 1940–1943, 1993.
Printed in Great Britain

0959–8049/93 $6.00 + 0.00

Cytotoxic Effect of Interferon on Primary Malignant Tumour Cells. Studies in Various Malignancies

Dan Grandér, Bo Xu and Stefan Einhorn

It is a well established fact that interferon (IFN) can inhibit cell growth, but only recently has it been found that IFN can exert a direct cytotoxic effect on primary tumour cells. This was shown in malignant cells from patients with multiple myeloma. In this study the influence of IFN on the viability of primary malignant cells from patients with different malignancies was studied. As previously described a direct cytotoxic effect of IFN on multiple myeloma cells was observed. No major effects on cell viability could be found in malignant cells from patients with lymphoma, chronic lymphocytic leukaemia, hairy cell leukaemia, chronic myelogenous leukaemia and carcinoma. This indicates that the direct cytotoxic effect of IFN in multiple myeloma may be relatively specific for this malignancy. It could be due to a specific differentiation stage in the myeloma cells, specific genetic alterations and/or abrogation of an autocrine/paracrine loop.
Eur J Cancer, Vol. 29A, No. 14, pp. 1940–1943, 1993.

INTRODUCTION

INTERFERONS (IFN) have been shown to induce remissions in a variety of malignancies [1]. The mechanisms behind IFN antitumour action are still unclear and may vary in different malignancies. Theoretically, IFN could act by an indirect host mediated mechanism or by a direct effect on the tumour cell, for instance by cell growth inhibition or by induction of differentiation (reviewed in [2]).

By the use of a dye exclusion assay we have previously shown that IFN can exert a direct cytotoxic effect, unrelated to cell growth inhibition, on primary malignant cells from patients with multiple myeloma [3, 4]. This may be one major reason why some patients with myeloma respond to IFN therapy. The exact mechanism behind the cytotoxic effect is still unknown.

In this study we have examined whether a similar effect could be obtained with IFN in other malignancies, and we have mainly focused our interest on other B cell malignancies. In most of the diseases tested, clinical studies have shown that IFN can induce remissions [1].

MATERIALS AND METHODS

Malignant cells

Bone marrow samples were obtained from 44 patients with multiple myeloma. 4 of the myeloma patients were on therapy with natural IFN-α (7×10^6 U/m^2/day for 5 consecutive days repeated every third week), but had not received IFN injections within 7 days prior to sampling. Peripheral blood samples were obtained from 6 patients with chronic lymphocytic leukaemia (CLL) and 1 patient with hairy cell leukaemia. Lymph nodes were obtained from 7 patients with B cell lymphomas (1 centroblastic, 1 centrocytic and 5 centroblastic/centrocytic lymphomas). Blood and bone marrow were obtained from 11 patients with chronic myelogenous leukaemia (CML). Ascites fluid was obtained from 3 patients with ovarian carcinoma. A surgical specimen from a patient with gastric cancer was also studied. None of the patients with malignancies other than myeloma were on IFN therapy at the time of sampling.

Preparation and culture of cells

Preparation of tumour cells from ascites, surgical specimens and lymph nodes has been described elsewhere [5]. Briefly, a cell suspension was prepared by treatment of finely minced tumour tissue with collagenase (3 mg/ml) and DNAse (0.2 mg/ml) for 30 min. Heparinised peripheral blood and bone marrow samples were centrifuged on a layer of Lymphoprep. All cells were subsequently collected and washed twice by centrifugation in medium (RPMI 1640 with 1% L-glutamine and 10% fetal calf serum for bone marrow cells from multiple myeloma patients and minimum essential medium, Eagle modified with 1% L-glutamine, 10% human AB-rhesus positive serum for all other cells). Subsequently, 1×10^6 cells were incubated for 4 days in round-bottomed 5-ml plastic tubes (Falcon 2058, Lincoln Park, New Jersey) with 1 ml of medium, at 37°C in the absence or presence of IFN (5000 U/ml), if not otherwise stated. For malignancies other than myeloma, the results obtained with

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Revised 28 Apr. 1993; accepted 11 June 1993.

lower doses of IFN-α (5 and 50 U/ml) were similar to the results obtained using 5000 U/ml (data not shown).

IFN preparations

Recombinant IFN-α_{2b} (from Schering-Plough) was derived from *E. coli*. The specific activity of this preparation was 2.0×10^8 U/mg of protein and the purity was >99%. Natural IFN-α was prepared from Sendaivirus-induced human Namalwa cells and purified by an anti-IFN-antibody affinity system [6]. The specific activity of this preparation was 2×10^8 U/mg of protein and the purity approximately 90%. Natural IFN-β was produced from fibroblasts by induction by poly(I) poly(C). The specific activity of this partially purified preparation was 1.6×10^6 U/mg of protein [7]. *E. coli*-derived recombinant human IFN-γ (from Ernst-Boehringer-Institut für Arznemittel Forschung) had a specific activity of 2×10^7 U/mg protein and a purity of >99%.

The antiviral activities of the preparations were determined by assaying inhibition of the cytopathic effect of vesicular stomatitis virus in human fibroblasts as described previously [8]. The antiviral activities are expressed in international units by comparison with international reference preparations.

Dye exclusion assay

A dye exclusion assay was used as previously described [9]. Briefly, a defined number of permanently fixed duck red blood cells (DRBC) was added to the cell suspensions after incubation, after which the cells were stained with 2% fast green and 1% of nigrosin dye solutions; 0.1 ml of both. After 10 min the cells were cytocentrifuged on to slides at 500 rpm for 10 min after which the slides were air dried and fixed with methanol for 20 s. The slides were counterstained with May-Grünwald-Giemsa (MGG) to enable differentiation between cell types. The DRBC served as an internal standard in this assay, minimising the problems with uneven distribution of the cells on the slides, cell autolysis and proliferation of the cells during incubation. Live malignant cells as well as live normal cells were calculated in relation to the DRBC in all slides. The number of viable cells in the cultures with IFN were expressed as a percentage of the number of viable cells in control cultures. Natural and recombinant IFN-α were used in different experiments in myeloma. In several control experiments natural and recombinant IFN-α were compared and found to give similar results in all cases. Due to this, the data in myeloma have been pooled. The results in some of the 44 patients with multiple myeloma have been published previously [3, 4].

To ascertain that the effect of IFN on the number of myeloma cells was a cytotoxic effect and not an effect on proliferation, autoradiographs were prepared for 3 patients to measure the proportion of normal and malignant cells entering S-phase. DNA synthesis was determined by the following procedure: a suspension of 10^6 cells/ml was incubated in the presence of 370 kBq [^{3}H] thymidine/ml in 1 ml of medium. The incubation time was 1–4 days. Autoradiography was performed with a liquid emulsion technique using Ilford K2 film. Following development the cells were stained with MGG. The labelling index was calculated by counting 500 cells and determining the percentage of cells containing >10 grains over the nucleus. In all cases studied, < 5% of the myeloma cells were labelled with [^{3}H]-thymidine during the culture period. During the same period IFN-α caused a reduction in myeloma cell number by up to 40% in these patients.

RESULTS

Multiple myeloma

Following incubation with IFN-α for 4 days a decrease in myeloma cell viability by ≥50% was seen in 21/44 patients (48%). In 3 of these cases less than 10% viable malignant cells remained after incubation. A 25–49% decrease was found in 8 patients, whereas IFN had no or only minor effects on the myeloma cells from 15 patients (Fig. 1). The mean reduction in myeloma cell number for all myeloma patients was 40 ± 6% (mean ± S.E.; $P<0.001$). Similar results were obtained with IFN-γ (Fig. 2) and IFN-β (data not shown). The effect of various concentrations of IFN-α was studied in cells from 5 patients. A reduction in cell viability was evident in some patients using 0.5 U/ml of IFN (data not shown). An optimal effect was seen at 5000 U/ml (data not shown).

In contrast to the results obtained with myeloma cells, the non-malignant bone marrow cells usually showed a low sensitivity to IFN-α. The mean decrease in viable cells after 4 days of culture was 18 ± 4% (mean ± S.E.). In contrast to myeloma cells the non-malignant cells were found to divide *in vitro*.

Lymphoma

Lymph node cells from 7 patients with lymphoma were incubated with α-IFN. In 1 case a 44% decrease in the viability of the malignant cells occurred subsequent to IFN treatment. In none of the other patients was any major decrease observed and the median number of viable malignant cells after IFN-α treatment was 107%, as compared with control cultures (Fig. 1). In 4 patients an increase in the amount of viable malignant cells was observed (Fig. 1).

In 5 patients the cells were also treated with IFN-γ. The results obtained were similar to those found with IFN-α (Fig. 2).

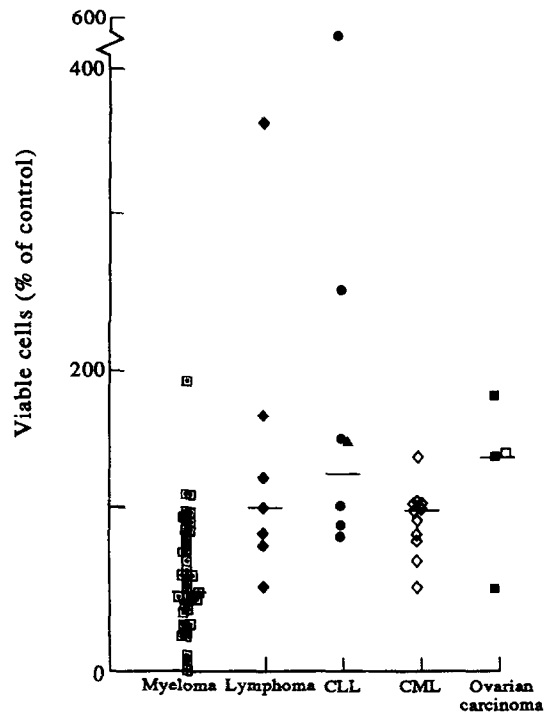

Fig. 1. Number of viable malignant cells following culture in the presence of 5000 U/ml of IFN-α for 4 days. The data are presented as a percentage of the number of viable cells cultured in the absence of IFN. The line denotes median values of samples from the different tumour groups. ▲ Hairy cell leukaemia, □ gastric carcinoma.

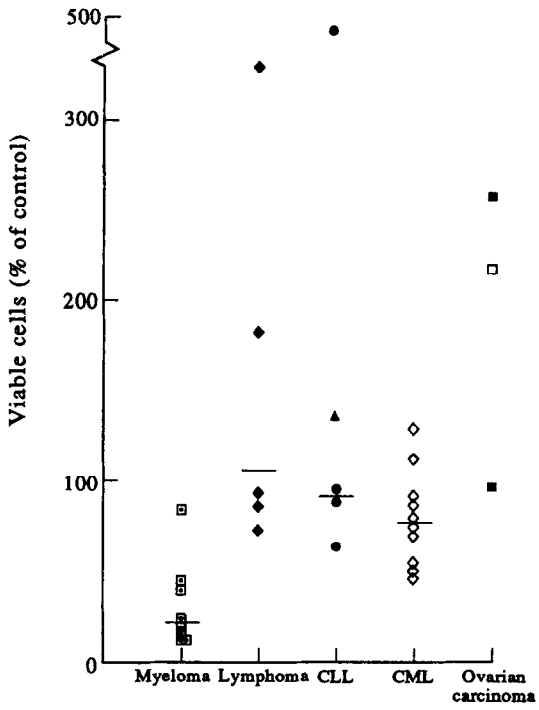

Fig. 2. Number of viable malignant cells following culture in the presence of 5000 U/ml of IFN-γ for 4 days. The data are presented as a percentage of the number of viable cells cultured in the absence of IFN. The line denotes median values of samples from the different tumour groups. ▲ Hairy cell leukaemia, □ gastric carcinoma.

CLL

In none of the 6 CLL patients was any major decrease in the viability of the malignant cells observed after incubation with IFN-α (Fig. 1). The median number of viable cells was 153%, as compared to control cultures. In 4 patients an increase in the amount of viable malignant cells was observed after IFN-α treatment (Fig. 1). Similar results were obtained following treatment with IFN-γ (Fig. 2). One case of hairy cell leukaemia was tested, in which the viability of the malignant cells was not decreased by either IFN-α (Fig. 1) or IFN-γ (Fig. 2).

CML

Peripheral blood cells from patients with CML were tested after culture with IFN-α (11 patients) or IFN-γ (10 patients). IFN-α had minor effects on cell viability (> 25% decrease) in 2 patients (Fig. 1). The mean decrease in cell viability was 2%.

In some CML cases IFN-γ had a slightly more pronounced effect on the cell number (Fig. 2). The mean decrease in the number of viable cells was 22%.

In 7 of the patients, bone marrow cells were also available for testing. The results obtained with both IFN-α and IFN-γ were similar to the results obtained using peripheral blood cells (data not shown).

Ovarian carcinoma

The IFN sensitivity of malignant cells from patients with ovarian carcinoma was tested in 3 cases. In 1 patient there was a reduction in cell viability by 45% with IFN-α, whereas no decrease in cell viability was seen in the other 2 patients (Fig. 1). In none of the patients did IFN-γ cause a decrease in cell viability (Fig. 2). No reduction in cell viability was seen in a patient with gastric carcinoma (Figs 1 and 2).

DISCUSSION

A number of previous studies have shown cell death subsequent to IFN treatment, but it has in most cases been hard to determine whether this is primarily due to cell growth inhibition or to a direct cytotoxic effect. One study has unequivocally shown that IFN can exert a cytotoxic effect in malignant cell lines [10].

We have previously shown that IFN treatment of primary myeloma cells, which are mainly non-dividing *in vitro*, leads to a significantly decreased viability of the myeloma cells [3, 4]. The effect does not seem to be mediated by immunologically active cells since, as we have previously shown, depletion of monocytes, granulocytes, T cells and natural killer cells does not abrogate the effect [3], indicating a direct cytotoxic effect of IFN on the myeloma cells. The exact mechanism behind this effect is still unclear, but it may be due to the induction of apoptosis in the myeloma cells, which has been shown to be the case in tumour necrosis factor-induced cytotoxicity in other tumour cells [11].

To determine whether a cytotoxic effect of IFN could also be shown for other primary malignant cells we studied the influence of IFN on cell viability in other malignancies, several of which have been shown to respond to IFN clinically. In none of the patient groups with malignancies other than multiple myeloma did we find any drastic reduction in the viability of the malignant cells, most patients being completely resistant (Figs 1 and 2). A cell growth inhibitory effect cannot be excluded in the few patients showing some sensitivity, since CML cells, for instance, can have a degree of cell division under the conditions used. In some patients an increased number of cells are observed after IFN treatment. The reasons for this may be several. One possibility is that IFN induces proliferation, as has been shown to be the case in malignant cells from some patients with CLL and lymphoma [12, 13].

These findings may indicate that the cytotoxic response seen in myeloma cells is an unusual, and possibly a fairly unique response of malignant cells to IFN. Naturally the findings in this study do not exclude the possibility that single patients with other malignant diseases may be highly sensitive to the cytotoxic effects of IFN, and that this effect may be responsible for the sometimes drastic antitumour effect observed in some diseases.

The reason for the sensitivity of myeloma cells to the cytotoxic effect of IFN may be their specific state of differentiation, and/or a specific combination of deregulated oncogenes/tumour suppressor genes. It may also be that IFN abrogates a specific autocrine/paracrine loop, as has been suggested for IL-6. We have found that IFN does not significantly affect the production of IL-6 in bone marrow cells from patients with multiple myeloma [14]. The possibility that IFN induces apoptosis in myeloma cells is currently being studied. An understanding of the mechanism behind IFN's cytotoxic effect in myeloma may help us in comprehending some aspects of IFN's antitumour action as well as give us tools for selecting patients with an increased chance of responding to IFN therapy.

1. Tyring SK. Introduction to clinical uses of interferons. In Baron S, Coppenhaver DH, Dianzani F, *et al*, eds. *Interferon: Principles and Medical Applications*. Galveston Texas, The University of Texas Medical Branch at Galveston, Department of Microbiology, 1992, 399–408.
2. De Maeyer E, De Maeyer-Guinard J. *Interferons and Other Regulatory Cytokines*. New York, John Wiley & Sons, 1988.
3. Einhorn S, Fernberg J-O, Grandér D, Lewensohn R. Interferon exerts a cytotoxic effect on primary human myeloma cells. *Eur J Cancer Clin Oncol* 1988, **24**, 1505–1510.

4. Grand D, von Stedingk LV, von Stedingk M, Wasserman J, Einhorn S. Influence of interferon on antibody production and viability of malignant cells from patients with multiple myeloma. *Eur J Haematol* 1991, **46**, 17–25.
5. Vanky F, Klein E, Willems J, Böök K, Ivert T, Pétreffy A. Recognition of autologous tumor cells by blood lymphocytes in patients with lung cancer. In Byers VS, Baldwin RW, eds. *Immunology of Malignant Diseases*. Lancaster, MTP Press Limited. 1987, 105–128.
6. Finter NB, Fantes KH. The purity and safety of interferons prepared for clinical use. The case for lymphoblastoid interferon. In Gresser I, ed. *Interferon*. New York, Academic Press, 1980, 65–80.
7. Obert HJ. Clinical trials and pilot studies with beta-interferon in Germany. In Merrigan TC, Friedman RM, eds. *UCLA Symposion on Molecular and Cellular Biology*. New York, Academic Press, 1982, Vol. 25, 426–432.
8. Einhorn L, Einhorn S, Wahren B. Interferon induction in human leukocytes after *in vitro* exposure to cytomegalovirus or Epstein-Barr virus. Influence of interferon on the expression of viral antigens. *Intervirology* 1985, **23**, 140–149.
9. Weisenthal LM, Marsden JA, Dill PL, Macaluso CK. A novel dye exclusion method for testing in vitro chemosensitivity of human tumors. *Cancer Res* 1983, **43**, 749–757.
10. Le J, Yip YK, Vilcek J. Cytolytic activity of interferon-gamma and its synergism with 5-fluorouracil. *Int J Cancer* 1984, **34**, 495–500.
11. Dealtry GB, Naylor MS, Fiers W, Balkwill FR. DNA fragmentation and cytotoxicity caused by tumor necrosis factor is enhanced by interferon-gamma. *Eur J Immunol* 1987, **17**, 689–693.
12. Östlund L, Einhorn S, Robèrt K-H, Juliusson G, Biberfeld P. Chronic B-lymphocytic leukemia cells proliferate and differentiate following exposure to interferon *in vitro*. *Blood* 1986, **67**, 152–159.
13. Östlund L, Robèrt K-H, Biberfeld P, Christensson B, Einhorn S. Induction of proliferation and blast transformation by interferon in human malignant and non-malignant lymph-node B-cells. *Blood* 1989, **73**, 2171–2181.
14. Grandér D. The interferon system in primary human tumor cells. Thesis, Karolinska Institute, 1991.

Acknowledgements—We would like to thank Elisabet Anderbring, Inger Bodin, Bo Carlsson, Ruth Detlofsson, Inger Foskolos and Marianne Lagnefeldt for their skilful technical assistance. Drs Farkas Vanky, Jan-Olof Fernberg, Anders Österborg, Karl Merk, Magnus Björkholm and Bengt Simonsson are thanked for their help in obtaining blood, bone marrow and tumour samples. This study was supported by grants from the Swedish Cancer Society and The Cancer Society of Stockholm.

Eur J Cancer, Vol. 29A, No. 14, pp. 1943–1947, 1993.
Printed in Great Britain

0959-8049/93 $6.00 + 0.00

Selection of Large and Objectively Measurable Target Lesions in EORTC Phase II Trials: Impact on Recruitment and Response Rate

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The EORTC has recently issued minimum requirements for target lesions in phase II trials, aiming at a decrease in measurement errors [minimum size, computer tomography (CT) scan or ultrasound for deep lesions]. Their impact on recruitment and response has been retrospectively studied in a trial of the EORTC Soft Tissue and Bone Sarcoma Group (STBSG), investigating high-dose chemotherapy in patients with advanced soft tissue sarcoma, where 46/103 objective responses were seen, including 10 complete responses. For the 20 patients who did not satisfy the criteria, a similar objective response rate and a significantly higher complete response rate were reported. Among 265 target lesions, the same trends were observed when comparing small to large lesions, for different tumour sites. For deep lesions clinically assessed, significantly higher response rates were reported than for those measured by CT scans or ultrasound. The new stricter EORTC criteria improve the reliability of measurements and have been adopted for future phase II trials of the STBSG. This will not result in the selection of potentially poor responders. Less than 20% of the present recruitment will be lost.
Eur J Cancer, Vol. 29A, No. 14, pp. 1943–1947, 1993.

INTRODUCTION

BECAUSE OF the number of compounds with potential activity against cancer, the methodology of screening and early development of new agents is important if the delay between discovery and application in large numbers of patients is to be minimised. At the clinical level, drugs with potential value are first evaluated in a limited number of patients for toxicity (phase I) and for antitumour activity (phase II). Only agents which have successfully completed these trials (i.e. the clinical screening phases) will be further developed and tested on a large scale (phase III). Phase II trials are crucial, because the decision to either terminate the development of a new agent, or invest large amounts of energy and money in its development and evaluation relies on their results.

Despite a seemingly well established methodology [1], comparison of published results of phase II trials often reveals important differences in response rates reported for a particular drug in a specific tumour type. Differences in the doses and schedules chosen for the trial, but also in the selection of the patients, are factors known to influence the conclusions of the trials [2–4]. Differences in response criteria play a role in this heterogeneity, but, surprisingly, critieria used for the evaluation are frequently not reported properly [5].