

Differential expression of interferon blocking activities in sera from patients with hepatocellular carcinoma

K. Karmaniolas¹, E. Papavasiliou², P. Ioannidis¹, Maria Dalamaga¹, S. Liatis¹, Efthimia Mandalaki¹, Th. Papalampros¹, I. Migdalis³

SUMMARY

Natural inhibitors to various cytokines including interferon (IFN), have been documented *in vitro* as well as *in vivo*. The aim of this study was to investigate the existence of IFN inhibitors in the serum of patients with hepatocellular carcinoma and to postulate their possible clinical significance. Sera from 16 patients with hepatocellular carcinoma, collected before any treatment, were tested while sera from 24 healthy blood donors were used as controls. All serum samples were tested for IFN- blocking and endogenous IFN-like activity. These activities were measured by a bioassay based on the IFN displaying antiviral state in three different cell lines highly sensitive to IFN: A₅₄₉, Intestine-407 and Chang liver cells. IFN-blocking activity was detected in the cell lines mentioned above in 93.7%, 12.5% and 37.5% of patients' sera respectively. No IFN-blocking activity was detected in the controls. The results support a cytokine and cytokine inhibitor network mediating pathophysiological events in the cellular level as well as in the whole organism. The limited responsiveness of hepatocellular carcinoma to rIFN α may potentially be due to the presence of such inhibitors.

Key Words: Interferon, Interferon inhibitors, Hepatocellular carcinoma, Cell sensitivity

ence of several inactivators and/or inhibitors in a variety of cultures, tissues and body fluids, including human sera.^{1,2} An antagonistic action between IFN, sarcolectins, growth factors and colony stimulating factors has also been documented in models *in vitro*.^{3,4} Furthermore, IFN is already known to act synergistically or antagonistically with tumor necrosis factor (TNF) and other cytokines, while natural inhibitors to various cytokines have also been described.⁵

In the last several years, extensive application of IFN and evaluation of its clinical potential in cancer and viral diseases has rekindled interest in the IFN inactivators and/or inhibitors (anti IFN activity). Interestingly, nonantibody type IFN inhibitors were found in the circulation of patients with neoplastic and viral diseases.^{6,7} The net biological effect would depend on the relative concentrations of various cytokines and inhibitors in the pericellular environment of any diseased tissue. IFN inhibitors have been implicated in IFN ineffectiveness during IFN treatment in patients with malignant neoplasia.^{2,7} The aim of this study was to investigate IFN inhibiting activity in sera from patients with hepatocellular carcinoma (a type of cancer not responding to IFN treatment), exerted in a variety of cellular types, as well as to elucidate the determinants of cellular sensitivity to such IFN inhibitors and/or antagonists.

INTRODUCTION

Early work on interferons (IFNs) revealed the exist-

¹1st Dept. of Internal Medicine, NIMTS Hospital, Athens, ²Gastroenterology Dept., NIMTS Hospital, Athens, ³2nd Dept. of Internal Medicine, NIMTS Hospital, Athens

Author for correspondence:

K. Karmaniolas, 69-71, Souidias str., 11521 Athens, Greece, Tel.: (+301) 7252784, Fax: (+301) 7293706

METHODOLOGY

Patients and controls

Sixteen patients with clinically evident hepatocellular carcinoma (HCC), diagnosed by physical examination, imaging and other laboratory examinations, were included in the study. The diagnosis was histologically confirmed in all cases. All patients, 14 men and 2 wom-

en, with a mean age of 62.4 years (range: 42-76 years), were hospitalized in NIMTS Hospital, Athens. Serum samples from all patients were collected before any kind of treatment and stored at -70° C until use. Sera controls were collected from 24 apparently healthy blood donors, with a mean age of 48.5 years (range: 40-58 years).

Method

IFN-inhibiting activity, as well as endogenous IFN-like activity, were determined in all serum samples in three established cell lines of different origin, cell type specificity and sensitivity to IFN: A₅₄₉ (ATCC, CCL 185, epithelial like cell line, lung carcinoma, human), Intestine-407 (Int-407, ATCC, CCL 6, epithelial like cell line, embryonic intestine, human) and Chang liver cells (ATCC, CCL 13, epithelial like cell line, liver, human).⁸

Assay of endogenous IFN activity: 50% inhibition of cytopathogenic effect (cpe) in a biological microculture system was used.^{9,10} A standard amount (0.2ml) of serum diluted 1:10 was added to each of four wells of a 96 well microplate. After incubation for 18-20 hours at 37° C in an atmosphere of 5% CO₂ for the establishment of the antiviral state, vesicular stomatitis virus (VSV) was added as a challenged virus (100 TCID₅₀/0.2ml/well). The development of virus specific cpe was observed after 24-48 hours. Inhibition of cpe indicates the presence of serum antiviral (protecting) activity in the cell cultures.

Assay of serum IFN-inhibiting activity: This activity was also determined in bioassay systems, based on the IFN antiviral activity.^{9,10} Briefly, equal volumes of serum dilutions and an optimum amount of rIFN- α (interferon alpha-2b, Intron A, Schering CO, USA) at 20u/ml were mixed and incubated for 1 hour at room temperature. Then, 0.2ml of the mixture was inoculated into 4 wells of a 96 well microplate for each of the 3 human epithelial-like cell lines. After incubation for 18-20 hours at 37°C in an atmosphere of 5% CO₂, VSV was added and incu-

bated again for 48 hours. Serum IFN-inhibiting activity was expressed as the reciprocal of serum dilution resulting in 50% cpe. Given that the final IFN concentration (10u/ml) protects control cell cultures (no virus cpe) 100%, all serum samples exhibiting IFN-inhibiting activity at a higher than 1:20 dilution were considered positive.

Concerning the statistical analysis of the results, χ^2 test was used. Concerning the comparison with zero frequency, the exact test of Fisher (LSD) was used.

RESULTS

There was no endogenous IFN-like activity in any of the patient group or control group. Our results on IFN-inhibiting activity of sera in the three cell lines used are summarized in Table 1. The IFN-blocking activity in A₅₄₉ cell cultures was expressed by a high percentage (93.7%) of sera ($p < 0.001$ vs. controls). In the Intestine-407 cell line only 2/16 sera expressed IFN-blocking activity, while in Chang liver cultures 6/16 sera exerted IFN-inhibiting activity ($p < 0.01$ vs. controls). In 24 serum samples from apparently healthy donors, the bioassay did not detect any anti-IFN activity.

DISCUSSION

According to our findings, there was no endogenous IFN-like activity in any patient or control group. These results agree with data from various studies, according to which, detection of endogenous IFN is not usual either in the sera of normal individuals¹¹ or in cancer patients' sera.^{12,13}

Concerning the IFN inhibitors, in our study, a high percentage of sera from patients with hepatocellular carcinoma exerted IFN-blocking activity in the A₅₄₉ cell line, which is a cell line highly sensitive to IFN. The well

Table 1. Incidence of sera from patients with hepatocellular cancer exerting IFN-blocking activity in 3 different cell lines

Sera with IFN-blocking activity in the cell line	A ₅₄₉	Int. 407	Chang-Liver
Hepatocellular cancer	*15/16 ¹ (93.7%)	2/16 (12.5%)	**6/16 (37.5%)
Controls	0/24	0/24	0/24

¹Numerator: number of sera bearing activity; denominator: number of sera tested

* $P < 0.001$ ** $P < 0.01$ VS controls

$P < 0.001$ A₅₄₉ VS Int-407

$P < 0.01$ A₅₄₉ VS Chang-Liver

$P < 0.05$ Int-407 VS Chang-Liver

known limited responsiveness that this type of cancer shows to IFN treatment might be due to the presence of these IFN inactivators. Recent studies confirm the presence of IFN inhibitors/antagonists in sera from cancer patients and verify our results.^{12,16}

Chadha et al¹⁴ reported the presence of IFN inhibitors in the serum of patients with ovarian cancer. The level of these inhibitors was reduced after successful irradiation or chemotherapy. Aszalos et al¹⁵ observed that sera from patients with AIDS, malignancies and SEL inhibited the IFN antiproliferative activity in Daudi cell cultures. This activity was due to the presence of IFN inhibitors in these. Tsantakis et al¹² observed IFN-inhibiting activity in sera from patients with hematological malignancies and solid tumors, while Karmaniolas et al¹³ observed a similar activity in sera from patients with lung cancer. Huschart et al¹⁶ also showed the presence of an IFN inhibiting factor (IIF) in patients suffering from multiple sclerosis and cancer.

The role of cell specificity (kind of cell culture used) in the IFN-blocking activity of cancer sera was really very important. Our findings support that there is a pattern of serum IFN-inhibiting activity which is expressed differentially in various biological systems. Among the tested lines, A₅₄₉ cells originating from lung cancer tissue, seem to be quite good responders to IFN-blocking activity of sera from hepatocellular carcinoma. On the contrary, the human embryonic cell line Intestine 407 and the Chang liver cells were not proved to be the best candidates in detecting cell type specific inhibitors of the IFN action in the case of HCC. Thus, Chang liver cells responded poorly, while IFN blocking activity in Int-407 cells was determined only in two cases ($p < 0.001$ A₅₄₉ vs. Int-407, $p < 0.01$ A₅₄₉ vs. Chang liver, $p < 0.05$ Int-407 vs. Chang liver). Different responsiveness, depending on the cell line used, was also observed in recent studies concerning other types of cancer.^{13,17} Thus, sera from lung cancer patients have been reported to show a different proportion of anti-IFN activity in three different types of cell lines.¹³ Moreover, sera from patients with solid tumors have also been reported to exert a different proportion of IFN-blocking activity in different biological systems.¹⁷

The nature and the role of such IFN-inhibiting activities are still an open field, since serum is rich in many kinds of non-specific as well as specific inhibitors.¹⁰ Even naturally occurring antibodies to IFN- α have been detected, although rarely, before any IFN treatment.¹⁸ The implication of non antibody type IFN-inhibitors has also been postulated to include a great number of probable

candidates: IL-6 activity,¹⁹ β 2 microglobulin and IgG or IgM immunoglobulins. Immunoglobulins have been reported to share anti-IFN activity and have been suggested to regulate cytokines, at least in part.²⁰ Prostaglandins have also been noted to modulate the ability of hyporeactive cells to respond to IFN-induction. Thus, soluble modulators such as prostaglandins, corticosteroids, etc., underscore both cytokine regulation and immune responsiveness.²¹

The hepatocellular carcinoma is considered to be a type of cancer which does not respond to IFN treatment. A systematic review of randomized controlled trials on HCC, mostly in non resectable patients, indicate that the non-surgical current treatments, including IFN treatment, are ineffective or minimally and uncertainly effective.²² Other studies indicate that IFN is not properly tolerated in patients with advanced HCC and that its administration prompts no benefit in terms of tumor progression rate and survival.²³ It seems that interferon regulatory factor - 1 gene abnormality is responsible for loss of growth inhibitory effect of IFN- α in human hepatoma cell lines.²⁴ On the other hand, interferon- α has been reported to have beneficial long term effects that reduce the occurrence of HCC in patients with chronic hepatitis C, even in those who do not have complete responses to IFN.²⁵ Other studies demonstrated that IFN- α would express growth suppression effects in human liver cancer cell lines, by inducing inhibition of cell-cycle progression with or without apoptosis, regardless of the expression level of Hu-IFN- α receptor protein on the cell surface.²⁶

The presented data from the cell-dependent IFN-inhibiting activities of patients' sera with hepatocellular cancer, further support an as yet unclear concept of cytokines and cytokine inhibitor network mediating auto-physiologic events. Further exploration of serum cytokine levels may reveal the clinical significance or may lead to the development of more specific and effective therapeutic schemes of IFNs, other cytokines and their inhibitors. Serum cytokine levels may therefore potentially be of clinical significance in follow-up of malignant diseases including hepatocellular carcinoma.

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