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# Pharmacokinetics, immune response, and biodistribution of iodine-131-labeled chimeric mouse/human IgG1, k 17-1A monoclonal antibody

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# Evidence for the Elevation of Serum Carcinoembryonic Antigen and Tumor-associated Glycoprotein-72 Levels in Patients Administered Interferons

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## ABSTRACT

**Sera were collected from 111 patients diagnosed with adenocarcinoma or nonadenocarcinoma malignancies who received different schedules of interferon (IFN)-γ or IFN-$\beta_{ser}$ alone or in combination. Serum carcinoembryonic antigen (CEA) and tumor-associated glycoprotein-72 (TAG-72) antigen levels were measured to determine whether interferon could enhance the tumor shedding and, thereby, the serum level of either tumor antigen. Less than 10% of the sera samples from patients diagnosed with nonadenocarcinoma malignancies (*e.g.*, hairy cell leukemia, melanoma) had positive titers of TAG-72 or CEA, and interferon neither increased nor resulted in the appearance of either tumor antigen in those sera. In contrast, 59.2% and 75.4% of the patients with adenocarcinoma had positive serum levels of TAG-72 and CEA, respectively, prior to interferon. IFN-γ and IFN-$\beta_{ser}$ alone or in combination significantly increased serum TAG-72 or CEA in approximately 65% of those patients. The results suggest that interferon administration to patients with adenocarcinoma can result in increased serum levels of selected tumor-associated antigens used in the diagnosis of malignancy. These preliminary findings may be important in the development of new strategies to obtain more sensitive tumor antigen serum assays for the diagnosis and monitoring for disease progression of adenocarcinoma.**

## INTRODUCTION

A part of clinical management of many human carcinomas is the measurement of serum tumor markers for the diagnosis of primary and/or recurrent lesions. Of the several tumor antigens used to monitor the course of disease for different carcinomas, CEA[2] is the best characterized. The presence of CEA in the serum of patients has been part of the clinical diagnosis of primary and recurrent gastrointestinal carcinoma (1, 2). In recent years, another human adenocarcinoma-associated antigen, TAG-72, has been well characterized (3–6); TAG-72 was initially described by the reactivity of MAb B72.3 to a human liver metastasis (7). Subsequent studies have shown that the TAG-72 antigen is a distinct glycoprotein of high molecular weight ($M_r > 10^6$) with the properties of a mucin (3, 5). A series of immunohistochemical studies have established that TAG-72 has wide distribution among different human carcinomas, *i.e.*, gastrointestinal, gynecological, non-small cell lung, mammary, and prostate (8–10), and previous studies have shown that both TAG-72 and CEA are noncoordinately expressed in carcinomas of the gastrointestinal tract (11, 12). The presence of TAG-72 in normal, adult tissues has, thus far, been limited to the secretory endometrium (13) as well as premalignant (14) and transitional mucosa in colon carcinoma patients (15). TAG-72 was purified from human colon carcinoma xenografts, and a series of "second generation" antibodies were produced using the purified glycoprotein as an immunogen (16). The availability of the second generation antibodies has led to the development of a double-determinant radioimmunoassay to detect the presence of TAG-72 in the sera and other fluids of carcinoma patients (17–20). Initial evaluation of the CA 72–4 assay was shown to detect the presence of TAG-72 in 35–40% of the sera from carcinoma patients (17). Using a cutoff value of 6 units/ml, 7–9% of patients with benign disease and 4% of healthy controls, respectively, had elevated serum TAG-72 levels (17, 18). More recently, studies have shown that complementarity exists for the presence of CEA and TAG-72 in the sera of patients with recurrent gastrointestinal carcinoma (21). These findings suggest that the measurement of both serum tumor antigens may be useful during the postsurgical follow-up in the ongoing management of those patients.

We have previously reported that certain human interferons can enhance the level of CEA and TAG-72 expression on the surface of a variety of human carcinoma cell populations and not on nonadenocarcinoma or normal cells which are constitutively negative for either antigen (22–24). Using CEA-specific complementary DNA probes, the ability of those cytokines to increase the level of expression of cell-associated CEA was accompanied by coordinate increases in CEA-related mRNA transcripts (25, 26). No CEA message was detected in normal cells either before or following interferon treatment. These findings suggest that the ability of interferon to increase CEA expression in human colon carcinoma cells includes changes at transcriptional and/or posttranscriptional sites that are involved in tumor antigen synthesis. In addition, experimental studies using human carcinoma cells freshly isolated from patients' malignant ascites have shown that incubation *in vitro* in the presence of either Type I (*i.e.*, IFN-α2a or IFN-$\beta_{ser}$) or Type II (*i.e.*, IFN-γ) interferon increases the level of expression of TAG-72 as measured by the binding of anti-TAG-72 MAbs (24). For comparison, cells isolated from the ascites of patients diagnosed with nonmalignant diseases (*i.e.*, inflammatory diseases) and/or nonadenocarcinomas (*i.e.*, melanoma, etc.) also did not express CEA or TAG-72 either before or after interferon treatment. Those studies provided the framework for the design of a clinical trial that reported substantial increases in the level of TAG-72 and CEA expression on human carcinoma cells isolated from ascites of patients given i.p. injections of IFN-γ.[4]

Recent studies also have demonstrated that IFN-α2a, IFN-$\beta_{ser}$, and IFN-γ treatment of selective human colon carcinoma cell lines as well as different human carcinoma cell populations isolated from malignant ascites can enhance the amount of CEA and TAG-72 released into the culture medium.[5] More-

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[2] The abbreviations used are: CEA, carcinoembryonic antigen; TAG-72, tumor-associated glycoprotein-72; IFN, interferon; MAb, monoclonal antibody; MU, million units.

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over, a clinical study designed to evaluate the effectiveness of IFN-γ as an antitumor agent has reported increased CA 19–9 in the sera of those patients (27). The present study utilized serum samples from a variety of clinical trials designed to determine the effectiveness of different interferons alone or in combinations for the treatment of malignancy. Sera were collected before and at various times after treatment with different interferons, and TAG-72 and CEA levels were measured to determine whether interferons could alter the serum levels of these tumor markers. The findings only represent a pilot study to determine whether interferon administration can affect serum CEA and TAG-72 levels since serum samples were collected from a variety of trials which administered different interferons on various dose schedules and by differing routes of injection. The results now provide evidence that a well-designed study should be carried out to define the various parameters (*i.e.*, dosage, interferon type, etc.) of interferon administration which will up-regulate serum CEA and TAG-72 levels in patients with confirmed and/or suspected carcinoma.

## MATERIALS AND METHODS

**Clinical Trials.** Table 1 lists the different protocols from which sera were collected prior to and after IFN administration in which TAG-72 and CEA levels were measured.

**Protocol A.** Three of the four patients received 3 escalating doses of IFN-γ i.v. that ranged from 0.1 to 800 MU; the fourth patient received four treatments which were escalated from 1 to 100 MU. Sera were collected prior to and at 1–4 days following treatment.

**Protocol B.** This was a single, high-dose IFN-γ trial. Each patient was administered 300 MU IFN-γ, and sera samples were collected prior to and 48 h after treatment.

**Protocol C.** Patients received daily i.m. (6 patients) or i.v. (2 patients) doses of 9 MU IFN-γ for 4–6 weeks. Sera samples were collected prior to, during, and immediately after treatment.

**Protocol D.** The dose schedule for the patients in the IFN-γ trial has been published (27). Initially, patients were given a 0.25-mg/m$^2$ i.v. injection daily for 10 days, which was discontinued due to severe side effects. Subsequently, all patients received i.m. injections of 0.25 mg/m$^2$ IFN-γ, and those patients not experiencing side effects were escalated to 0.5 mg/m$^2$ on day 8. Serum samples were taken prior to IFN-γ treatment and on days 2, 10, and 29 (27).

**Protocol E.** Patients in protocol E received a daily i.m. injection of 0.1 mg/m$^2$ of IFN-γ for 15 days (28). Serum samples were taken from each patient before IFN-γ therapy (day 1) and on days 7, 15, and 23.

**Protocol F.** Patients received 90 MU IFN-$\beta_{ser}$ and, 7 days later, 450 MU, and sera were collected at 24 and 48 h after each treatment.

**Protocol G.** Patients in protocol G were given 2.64–26.4 MU IFN-$\beta_{ser}$ i.v. on day 1 and received subsequent daily s.c. injections on days 8–28. Serum samples were drawn at 24 and 48 h posttreatment.

**Protocol H.** Patients diagnosed with hairy cell leukemia were administered a single s.c. injection of IFN-$\beta_{ser}$, and serum samples were removed prior to and 24 h after treatment.

**Protocol I.** The details of the dose schedule for protocol I have been published (29). Patients received 45 or 180 IU IFN-$\beta_{ser}$ i.v., and sera were collected before and 24 h after treatment. Doses and routes were randomly assigned to four patient groups. Patients received either 45 or 180 IU IFN-γ on day 1 and the other dose on day 8. Patients began therapy on day 10 with 180 × 10$^6$ IU i.v. three times a week.

**Protocol J.** This was a high-dose IFN-$\beta_{ser}$ trial. During the first week, patients received three treatments of 90 MU followed by weekly administration of 450 MU. Sera samples were collected prior to and at 24 h after 90 MU IFN-$\beta_{ser}$ and prior to and at 24 and 48 h after 450 MU.

**Protocol K.** Patients received 3 MU IFN-$\beta_{ser}$ i.v. daily for 14 days, which was repeated every month; serum samples were collected prior to IFN-$\beta_{ser}$ and after 72 h of treatment.

**Protocol L.** The complete explanation for the dose schedule of protocol L has been published (30). Two dose levels were given weekly for 3 weeks (days 1, 8, and 15): a low dose of 3 MU of IFN-$\beta_{ser}$ and 200 μg IFN-γ; and a high dose which was a 10-fold increase in both interferons. Administration of IFN-γ i.v. over 10 min was followed by a 10-min injection of IFN-$\beta_{ser}$. Sera were collected before and 24 h after interferon treatment and after 4 weeks of interferon maintenance therapy of IFN-$\beta_{ser}$/γ 3 times per week.

**Protocol M.** The dose schedules for protocol M included the administration of IFN-$\beta_{ser}$ and IFN-γ in combination by continuous i.v. infusion for 5 days (31).

**Serum Samples.** Serum samples from the 13 different clinical trials listed in Table 1 were stored at −20°C. The samples were thawed immediately prior to the measurement of CEA and TAG-72 antigen concentrations.

**Measurement of CEA and TAG-72 Levels.** CEA levels were measured using the CEA radioimmunoassay monoclonal *in vitro* test kit purchased from Abbott Laboratories, Inc. (Chicago, IL). Positive serum CEA values were those 5.0 ng/ml or higher. Serum TAG-72 was measured using the double-determinant radioimmunoassay (CA 72–4; Centocor, Inc., Malvern, PA) (17, 18). The cutoff value for positive serum TAG-72 levels was 6.0 units/ml. Briefly, 100 μl of specimen and 100 μl of phosphate buffer were added in duplicate to polystyrene beads coated with MAb CC49. The solution was incubated for 4 h at 37°C, and the beads were washed, after which $^{125}$I-labeled B72.3 was added and incubated for 18 h at 4°C. Bound radioactivity was measured in a gamma scintillation counter, and the cpm were converted to concentration values (*i.e.*, units/ml) using a concurrently obtained standard curve. Samples with TAG-72 or CEA levels above that for each respective standard curve were reassayed using the appropriate dilutions. In all assays, internal low and high standards for CEA or TAG-72 were included.

**Statistical Analysis of Serum Tumor Antigen Determinations.** In a previous study, daily changes in serum CEA levels were evaluated in 55 patients diagnosed with advanced cancer (32). Patients were divided into those with stable, progressive, or regressive disease. Among those with stable disease, there was a 95% degree of confidence that daily CEA values for up to 30 days would be within 36% of the initial baseline value. In the present clinical protocols, no clinical responses were observed; however, most patients were diagnosed with stable disease during the interval of interferon administration. Therefore, increases or decreases of serum CEA values >36% of the initial value were considered changes associated with interferon administration. In lieu of a similar analysis for serum TAG-72 levels, it was decided to also consider changes in serum TAG-72 levels which exceed 36% as evidence of an interferon-mediated increase or decrease in antigen secretion.

## RESULTS

Table 1 lists the 13 different protocols from which sera of patients diagnosed with adenocarcinomas or other malignancies were collected and analyzed for TAG-72 and CEA levels prior to and following interferon administration. Most protocols utilized either IFN-γ or IFN-$\beta_{ser}$ as single agents with different dose schedules. Two protocols (L and M) administered IFN-γ and IFN-$\beta_{ser}$ sequentially, and the dose schedules have been published previously (30, 31). Both TAG-72 and CEA are used primarily as serum markers for patients diagnosed with adenocarcinoma (2, 17, 18). Therefore, for data analyses, patients were grouped with either adenocarcinoma or other tumors. Other tumors included 30 patients diagnosed with nonadenocarcinoma malignancies (*i.e.*, renal cell, melanoma, and others) as well as 5 patients with hairy cell leukemia (Table 2). Table 2 summarizes the number of patients within each group with positive serum TAG-72 and CEA levels as well as the range of values for each tumor antigen (shown in parentheses). These data established the constitutive levels of serum TAG-72 and

Table 1 *Summary of the therapeutic interferon clinical protocols used in the analysis of serum CEA and TAG-72 levels*

| Clinical protocol | Patient diagnosis[a] | Interferon treatment | Dose schedule |
|---|---|---|---|
| A | Breast cancer (2)<br>Colorectal cancer (1)<br>Thyroid cancer (1) | IFN-$\gamma$ | Intrapatient escalation 0.1–100 MU |
| B | Colorectal cancer (1)<br>Ovarian cancer (1) | IFN-$\gamma$ | Single treatment, 300 MU/m$^2$ |
| C | Lung cancer (non-small cell) (3)<br>Breast cancer (1)<br>Bladder cancer (1)<br>Esophagus cancer (1)<br>Renal cell cancer (1)<br>Stomach cancer (1) | IFN-$\gamma$ | 9 MU/day, 4–6 weeks |
| D | Colorectal cancer (26) | IFN-$\gamma$ | 0.25 mg/m$^2$, i.m., 7 days: day 8 escalated to 0.5 mg/m$^2$ for 30 days |
| E | Colorectal cancer (15) | IFN-$\gamma$ | 0.1 mg/m$^2$ for 15 days (Ref. 28) |
| F | Colorectal cancer (4) | IFN-$\beta_{ser}$ | Intrapatient escalation 90, 450 MU |
| G | Unknown cancer (2)<br>Renal cell cancer (5)<br>Melanoma (3)<br>Breast cancer (1)<br>Carcinoid (1) | IFN-$\beta_{ser}$ | Single i.v., daily s.c. 2.64–26.4 MU |
| H | Hairy cell leukemia (5) | IFN-$\beta_{ser}$ | Single injection, 90 MU |
| I | Renal cell cancer (1)<br>Breast cancer (1) | IFN-$\beta_{ser}$ | 45–180 MU weekly (Ref. 29) |
| J | Melanoma (3) | IFN-$\beta_{ser}$ | 90–450 MU × 10$^6$ IU 3 times per week, escalation every 2 weeks |
| K | Breast cancer (1)<br>Colorectal cancer (1)<br>Uterine cancer (1) | IFN-$\beta_{ser}$ | 3 MU i.v. for 14 days once a month |
| L | Colorectal cancer (3)<br>Breast cancer (2)<br>Pancreas cancer (2)<br>Lung cancer (non-small cell) (2) (large cell) (1) | IFN-$\beta_{ser}/\gamma$ | Weekly treatment (2 dose schedules) (Ref. 30) |
| M | Thymus cancer (1)<br>Lung cancer (2)<br>Renal cell cancer (6)<br>Melanoma (6)<br>GE-junction (1) | IFN-$\beta_{ser}/\gamma$ | 5-day continuous infusion (Ref. 31) |

[a] Numbers in parentheses, number of patients.

Table 2 *Serum CEA and TAG-72 levels prior to interferon in patients diagnosed with adenocarcinomas and nonadenocarcinoma tumors*

| Diagnosis | Number of patients | Antigen positive serum profile | |
|---|---|---|---|
| | | TAG-72 (units/ml) | CEA (ng/ml) |
| Adenocarcinomas | | | |
| Gastrointestinal | 54 | 37 (6.5–351)[a] | 36 (5.3–13,400)[b] |
| Lung | 8 | 3 (7.1–24) | 5 (7.5–663) |
| Breast | 8 | 2 (16.4 and 31.3) | 3 (7-841) |
| Unknown | 2 | 2 (282 and 567) | None |
| Others[c] | 4 | 1 (175) | 2 (27.7 and 504) |
| Totals | 76 | 45 (59.2%) | 46 (75.4%)[b] |
| Other tumors | | | |
| Renal cell | 13 | None | None |
| Melanoma | 12 | 1 (8.4) | None |
| Hairy cell leukemia | 5 | None | 1 (8.1) |
| Others[d] | 5 | 2 (6.3 and 6.6) | 1 (863) |
| Totals | 35 | 3 (8.6%) | 2 (5.7%) |

[a] Numbers in parentheses, range of antigen units in each group.
[b] CEA determinations were not done on the 15 sera from patients diagnosed with advanced colorectal carcinoma (protocol E) due to insufficient amount of serum. Therefore, of the sera samples from the remaining 61 patients, 46 (75.4%) were positive.
[c] Others include adenocarcinoma of the pancreas ($n$ = 2), ovarian ($n$ = 1), and uterus ($n$ = 1).
[d] Other tumors include bladder ($n$ = 1), thymus ($n$ = 1), thyroid ($n$ = 1), carcinoid ($n$ = 1), and head and neck ($n$ = 1).

Table 3 *Effects of interferon on the serum levels of TAG-72 and CEA in patients with nonadenocarcinoma malignancies*

| Interferon protocol | Diagnosis | Serum antigen levels | | | |
|---|---|---|---|---|---|
| | | TAG-72 (units/ml) | | CEA (ng/ml) | |
| | | Pretreatment | Post-IFN | Pretreatment | Post-IFN |
| A | Thyroid | Neg[a] | Neg | 863.1 | 797.1 |
| C | Bladder | Neg | Neg | Neg | Neg |
| | Renal | Neg | Neg | Neg | Neg |
| G | Renal | Neg | Neg | Neg | Neg |
| | Renal | Neg | Neg | Neg | Neg |
| | Renal | Neg | Neg | Neg | Neg |
| | Renal | Neg | Neg | Neg | Neg |
| | Renal | Neg | Neg | Neg | Neg |
| | Melanoma | Neg | Neg | Neg | Neg |
| | Melanoma | Neg | Neq | Neg | Neg |
| | Melanoma | Neg | Neg | Neg | Neg |
| | Head and Neck | 6.3 | 7.6 | Neg | Neg |
| | Carcinoid | Neg | Neg | Neg | Neg |
| H | Hairy cell leukemia | Neg | Neg | Neg | Neg |
| | Hairy cell leukemia | Neg | Neg | Neg | Neg |
| | Hairy cell leukemia | Neg | Neg | 8.1 | 9.8 |
| | Hairy cell leukemia | Neg | Neg | Neg | Neg |
| | Hairy cell leukemia | Neg | 13.8 | Neg | Neg |
| M | Thymus | 6.6 | 7.9 | Neg | Neg |
| | Renal | Neg | Neg | Neg | Neg |
| | Renal | Neg | Neg | Neg | Neg |
| | Renal | Neg | Neg | Neg | Neg |
| | Renal | Neg | Neg | Neg | Neg |
| | Renal | Neg | Neg | Neg | Neg |
| | Renal | Neg | Neg | Neg | Neg |
| | Melanoma | Neg | Neg | Neg | Neg |
| | Melanoma | Neg | Neg | Neg | Neg |
| | Melanoma | Neg | Neg | Neg | Neg |
| | Melanoma | 8.4 | Neg | Neg | Neg |
| | Melanoma | Neg | Neg | Neg | Neg |
| | Melanoma | Neg | Neg | Neg | Neg |
| I | Renal | Neg | Neg | Neg | Neg |
| J | Melanoma | Neg | Neg | Neg | Neg |
| | Melanoma | Neg | Neg | Neg | Neg |
| | Melanoma | Neg | Neg | Neg | Neg |
| Totals | 35 | 3 (8.6%) | 3 | 2 (5.7%) | 2 |

[a] Neg, negative.

CEA prior to interferon administration. As shown, 54 of 76 (71.1%) patients who were diagnosed with adenocarcinoma had tumors of the gastrointestinal tract. Thirty-seven of those 54 patients (68.5%) had positive serum TAG-72 levels prior to the initiation of interferon-based therapy. Likewise, 36 of 39 patients (92.3%) diagnosed with adenocarcinoma of the gastrointestinal tract had positive serum CEA (Table 2). Combining all patients diagnosed with adenocarcinoma, positive serum TAG-72 levels were found in 45 of 76 (59.2%) patients, and positive CEA levels were measured in 46 of 61 (75.4%) patients, respectively (Table 2). In contrast, only 3 (8.6%) and 2 (5.7%) of 35 patients diagnosed with other tumors that included renal cell carcinoma, melanoma, hairy cell leukemia, etc. had positive serum TAG-72 or CEA, respectively.

Sera collected from patients diagnosed with nonadenocarcinoma malignancies were analyzed to determine whether interferon administration would induce the appearance of TAG-72 and/or CEA or increase the constitutive level of either serum tumor antigen (Table 3). As shown, >90% of the sera analyzed from patients diagnosed with those tumor types (*i.e.*, thyroid, bladder, renal cell, melanoma, head and neck, carcinoid, and hairy cell leukemia) did not contain measurable levels of either TAG-72 (32 of 35) or CEA (33 of 35) prior to or following interferon treatment. Five patients in which serum TAG-72 or CEA levels were positive prior to interferon administration had no subsequent increase in the level of either tumor antigen following treatment with the cytokine. The serum sample collected from a patient diagnosed with malignant melanoma (protocol M) contained 8.4 units TAG-72/ml prior to interferon administration; however, after treatment serum TAG-72 levels had fallen below the cutoff. Another patient diagnosed with hairy cell leukemia had positive serum TAG-72 levels following 2 successive days of IFN-$\beta_{ser}$ ($90 \times 10^6$ units) treatment (protocol H). Therefore, only a small percentage of patients diagnosed with nonadenocarcinoma malignancies had positive serum levels of either TAG-72 or CEA. Interferon treatment, furthermore, neither resulted in the appearance of either tumor antigen nor dramatically increased the serum TAG-72 or CEA in the five patients with measurable titers of either antigen.

The largest number of patients who were evaluated for changes in serum tumor antigen levels were the 26 patients from protocol D, who were diagnosed with advanced colorectal cancer. Patients received 0.25 mg/m$^2$ IFN-$\gamma$ which was escalated to 0.5 mg/m$^2$ on day 8, and serum TAG-72 and/or CEA levels were measured prior to and at days 1, 2, 10, and 30 during daily IFN-$\gamma$ administration. As outlined earlier, changes in TAG-72 expression of >36% were considered indicative of increased release of the tumor antigen as a result of interferon administration. Prior to IFN-$\gamma$ treatment the mean serum TAG-72 was 48.1 units/ml (Table 4). As early as 2 days post-

Table 4 *Serum levels of TAG-72 in patients receiving IFN-γ for colorectal cancer*

| Days on study | Serum TAG-72 (units/ml) | | |
|---|---|---|---|
| | *n* | Mean | Minimum/ maximum |
| Pre-IFN-γ | 26 | 48.1 | 6.5/351 |
| 2 | 24 | 69.5 (44.5)[a] | 6.1/664 |
| 10 | 23 | 97.1 (102.1) | 6.9/887 |
| 30 | 19 | 256.3 (432.8) | 6.7/3,770 |

[a] Numbers in parentheses, percentage increase in the mean serum TAG-72 levels above that measured in the pre-IFN-γ samples. This was calculated as:

$$\frac{\text{Serum TAG-72 day 2, 10, or 30} - \text{serum TAG-72 (pre-IFN-}\gamma\text{)}}{\text{Serum TAG-72 (pre-IFN-}\gamma\text{)}} \times 100$$

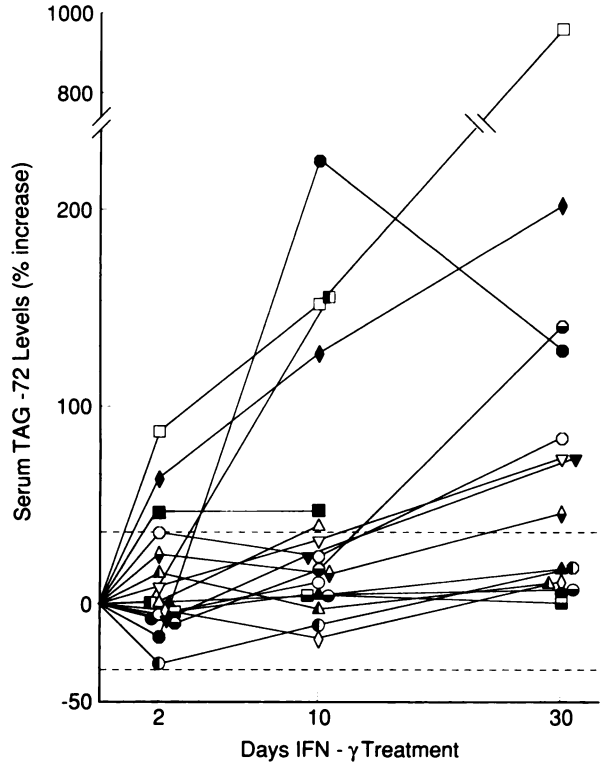


Fig. 1. Changes in serum TAG-72 levels in patients diagnosed with colorectal adenocarcinoma and treated with IFN-γ (protocol D) (27). The different lines represent % increase above control in serum TAG-72 levels in 19 patients who had measurable serum antigen prior to IFN-γ administration. The *dotted lines* indicate ±36% from the initial (*i.e.*, pre-IFN-γ) serum TAG-72. As stated previously, a <36% increase in serum TAG-72 levels was considered to be the result of increased antigen release by the tumor. The changes in serum TAG-72 levels were calculated as indicated in Table 4.

interferon treatment, the serum TAG-72 levels had increased by 44.5% to 69.5 units/ml. After 10 and 30 days of IFN-γ treatment, serum TAG-72 levels had increased to 97.1 and 256.3 units/ml, respectively, values that are 102.1% and 432.8% of the pre-IFN-γ levels. Of the initial 26 patients, longitudinal changes in serum TAG-72 levels were followed in 19 patients for 30 days (Fig. 1). The serum levels of TAG-72 were elevated by >36% for 11 of the 19 patients (57.9%) during IFN-γ treatment. It should be noted that none of the patients showed a significant drop in serum TAG-72 levels during IFN-γ treatment, and six of the eleven patients who had a >36% increase in serum TAG-72 were increased after 10 days. Fig. 2 summarizes the relative rates of change for serum TAG-72 and CEA during a 30-day treatment with IFN-γ, suggesting that changes induced in serum TAG-72 may increase at a faster rate than those in CEA.

Tables 5 and 6 summarize the changes observed in serum TAG-72 and CEA, respectively, grouped according to whether the clinical protocol included the administration of IFN-γ or IFN-$\beta_{ser}$ alone or in combination. Of the 76 patients diagnosed with adenocarcinoma, 52 received IFN-γ alone (protocols A-E). The sera samples from 30 of the 52 patients (57.5%) prior to IFN-γ administration were positive for TAG-72, and IFN-γ treatment increased serum TAG-72 in 17 of the 30 cases (56.7%). The administration of IFN-$\beta_{ser}$ alone or in combination with IFN-γ increased serum TAG-72 levels in 80.0% of the patients with positive TAG-72 serum levels (Table 5). As shown in Table 6, of the 37 patients diagnosed with adenocarcinoma (*i.e.*, 15 patients from protocol E were not evaluated for serum CEA), 32 (86.5%) had positive serum CEA levels, and IFN-γ treatment increased serum CEA in 26 of the 32 cases (81.3%). Eight patients whose sera samples contained positive CEA levels received either IFN-$\beta_{ser}$ alone or in combination with IFN-γ, and serum CEA levels were increased in 2 of 8 and 5 of 8 cases, respectively (Table 6).

Tables 7 and 8 summarize the effects of interferon treatment on serum TAG-72 and CEA levels, respectively, from patients diagnosed with different adenocarcinomas. Most of the data were generated from patients diagnosed with adenocarcinoma of the gastrointestinal tract. Therefore, it is impossible to determine whether interferon preferentially increases serum TAG-72 and/or CEA levels from selective types of carcinoma. In any case, sera analyzed from 44 of the 76 (57.9%) patients diagnosed with adenocarcinomas contained positive titers of TAG-72, and interferon administration increased the level of that tumor marker in 29 of the 44 cases (65.9%). Interestingly, of 32 patients who did not have positive TAG-72 serum levels before interferon administration, 7 (21.9%) had measurable serum TAG-72 levels after treatment (Table 7). Table 8 summarizes the changes induced in serum CEA levels in patients diagnosed with different adenocarcinomas and treatment with

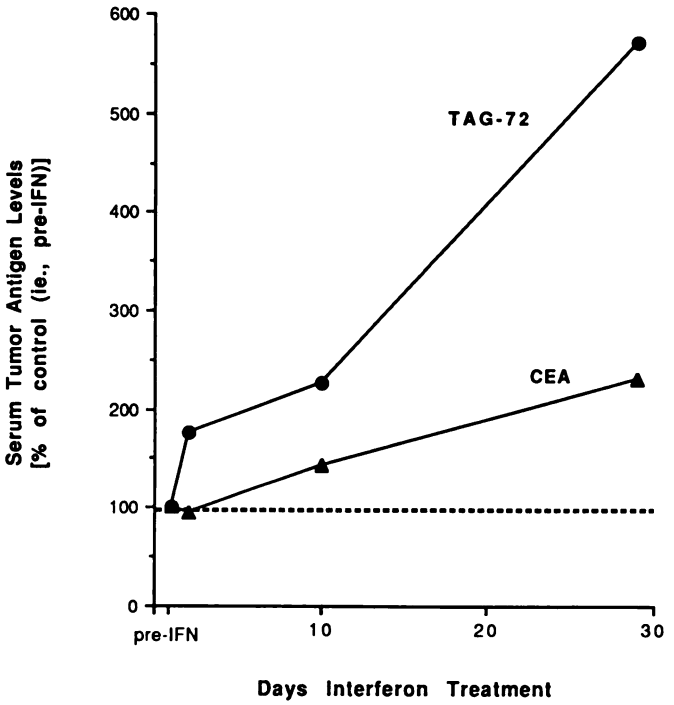


Fig. 2. Comparison of the time-dependent changes in serum TAG-72 (●) and CEA (▲) levels in patients treated with IFN-γ (protocol D). *Points*, means of TAG-72 and CEA levels in sera samples collected from patients prior to and at days 2, 10, and 30 days after IFN-γ treatment. The values for serum CEA levels were taken from a previously published report (27).

Table 5 *Summary of the effects of IFN-γ, IFN-$\beta_{ser}$, and a combination of IFN-$\beta_{ser}$ and IFN-γ on serum TAG-72 levels*

| Clinical protocol | Number of adenocarcinoma patients | Number of TAG-72-positive | IFN-treatment-increased TAG-72 |
|---|---|---|---|
| IFN-γ treatment | | | |
| A | 2 | 0/2 (0%)[a] | NA[b] |
| B | 2 | 1/2 (50%) | 0/1 (0%)[c] |
| C | 7 | 2/7 (28.6%) | 2/2 (100%) |
| D | 26 | 19/26 (73.1%) | 11/19 (58%) |
| E | 15 | 8/15 (53.3%) | 4/8 (50%) |
| Totals | 52 | 30/52 (57.7%) | 17/30 (56.7%) |
| IFN-$\beta_{ser}$ treatment | | | |
| F | 4 | 2/4 (50%) | 2/2 (100%) |
| G | 3 | 2/3 (67%) | 2/2 (100%) |
| H | None | NA | NA |
| I | 1 | 1/1 (100%) | 0/1 (0%) |
| J | None | NA | NA |
| K | 3 | 0/3 (0%) | NA |
| Totals | 11 | 5/11 (45.4%) | 4/5 (80.0%) |
| IFN-$\beta_{ser}$/γ treatment | | | |
| L | 10 | 3/10 (30.0%) | 2/3 (67%) |
| M | 3 | 2/3 (66.6%) | 2/2 (100%) |
| Totals | 13 | 5/13 (38.5%) | 4/5 (80.0%) |

[a] Numbers in parentheses, percentage of the patients diagnosed with carcinoma and having >6.0 units TAG-72/ml in their serum.
[b] NA, not applicable.
[c] Numbers in parentheses, percentage of patients whose serum TAG-72 levels increased by >36% with interferon treatment.

Table 6 *Summary of the effects of interferon on serum CEA levels*

| Clinical protocol | Number of adenocarcinoma patients | Number of CEA-positive | IFN-treatment-increased CEA |
|---|---|---|---|
| IFN-γ treatment | | | |
| A | 2 | 2/2 (100%)[a] | 2/2 (100%) |
| B | 2 | 2/2 (100%) | 2/2 (100%) |
| C | 7 | 4/7 (57.1%) | 4/4 (100%) |
| D | 26 | 24/26 (92.3%) | 18/24 (75.0%) |
| E | 15 | ND[b] | ND |
| Totals | 37[c] | 32/37 (86.5%) | 26/32 (81.3%) |
| IFN-$\beta_{ser}$ treatment | | | |
| F | 4 | 4/4 (100%) | 1/4 (25%) |
| G | 3 | 1/3 (33.3%) | 0/1 (0%) |
| H | None | NA | NA |
| I | 1 | 0/0 (0%) | NA |
| J | None | NA | NA |
| K | 3 | 3/3 (0%) | 1/3 (33.3%) |
| Totals | 11 | 8/11 (72.7%) | 2/8 (25.0%) |
| IFN-$\beta_{ser}$/γ treatment | | | |
| L | 10 | 5/10 (50.0%) | 4/5 (80%) |
| M | 3 | 3/3 (100%) | 1/3 (33.3%) |
| Totals | 13 | 8/13 (61.5%) | 5/8 (62.5%) |

[a] See Table 5 for an explanation of numbers in parentheses.
[b] ND, not determined; NA, not applicable.
[c] Does not include the 15 patients from protocol E serum; CEA values were not measured for those patients.

Table 7 *Summary of the changes in serum TAG-72 levels following interferon treatment*

| Type of carcinoma | Number of patients | Cumulative patient serum TAG-72 profile | | | |
|---|---|---|---|---|---|
| | | Number of TAG-72-positive | Number of increased post-IFN | Number of TAG-72-negative | Number of TAG-72-positive post-IFN |
| Gastrointestinal | 54 | 36 | 24 (66.6%)[a] | 18 | 5 (27.8%) |
| Lung | 8 | 3 | 3 (100%) | 5 | 2 (40.0%) |
| Breast | 8 | 2 | None | 6 | None |
| Unknown | 2 | 2 | 2 (100%) | NA[b] | NA |
| Others[c] | 4 | 1 | None | 3 | None |
| Totals | 76 | 44 (57.9%) | 29 (65.9%) | 32 (41.6%) | 7 (21.9%) |

[a] Numbers in parentheses, percentage of patients with TAG-72-positive or -negative serum levels which are the result of interferon-treatment-increased TAG-72 serum levels.
[b] NA, not applicable.
[c] Others include adenocarcinoma of the pancreas ($n$ = 2), ovary ($n$ = 1), and uterus ($n$ = 1).

Table 8 *Summary of the changes in serum CEA levels following interferon treatment*

| Type of carcinoma | Number of patients | Cumulative patient serum CEA profile | | | |
|---|---|---|---|---|---|
| | | Number of CEA-positive | Number of increased post-IFN | Number of CEA-negative | Number of CEA-positive post-IFN |
| Gastrointestinal | 39 | 36 | 22 (61.1%)[a] | 3 | 1 (33.3%) |
| Lung | 8 | 5 | 3 (100%) | 3 | None |
| Breast | 8 | 3 | 3 (100%) | 5 | 1 (20.0%) |
| Unknown | 2 | None | None | 2 | None |
| Others[b] | 4 | 2 | 2 (100%) | 2 | None |
| Totals | 61 | 46 (75.4%) | 30 (65.2%) | 15 (24.6%) | 2 (13.3%) |

[a] See Table 7 for explanation of numbers in parentheses.
[b] Others include adenocarcinoma of the pancreas ($n$ = 2), ovary ($n$ = 1), bladder ($n$ = 1), and uterus ($n$ = 1).

interferon. CEA levels were detected in 46 of 61 (75.4%) of the patients diagnosed with adenocarcinoma, and interferon administration resulted in an increase in antigen serum content in 30 of the 46 cases (65.2%) (Table 8). Of the 15 cases in which serum CEA was negative, interferon treatment resulted in the appearance of positive CEA titers in 2 cases (13.3%).

## DISCUSSION

The recombinant interferons have previously been shown to up-regulate the level of expression of selected human tumor antigens, such as TAG-72 and CEA, on the surface of a variety of human carcinoma cells (22–27, 34). Experimental data have shown that the increase in CEA expression is accompanied by increases in CEA-related mRNA transcripts, and these changes result in enhanced binding of anti-CEA MAbs to the tumor cell surface (25, 26). The ability to alter the tumor antigen phenotype on the surface of human carcinoma cells may be exploited to improve the localization of a conjugated MAb *in vivo* (33, 34), and it has been postulated that this could improve the diagnostic and therapeutic effectiveness of the MAb (34, 35). In fact, a clinical study has been completed that demonstrated substantial increases in the level of TAG-72 and CEA expression on human carcinoma cells in malignant ascites following the i.p. administration of IFN-$\gamma$.[3] Recently, data generated in both experimental and clinical investigations have suggested that the interferons could also increase the secretion of TAG-72 and CEA from human carcinoma cell populations. Using either established human tumor cell lines or carcinoma cells isolated from malignant ascites, treatment with either Type I or Type II interferons increased the amount of CEA and/or TAG-72 shed into the medium.[4] A recent clinical trial reported that the administration of IFN-$\gamma$ at doses used to evaluate its potential antitumor activity were found to increase the serum level of CA 19–9 and, to a lesser extent, CEA (27). Therefore, it was decided to analyze sera from different clinical trials in which patients diagnosed with adenocarcinoma or nonadenocarcinoma tumors and treated with either Type I and/or Type II interferons, as a first step to determining whether cytokine treatment could bring about changes in the serum levels of specific human tumor antigens.

Sera were collected from 13 different clinical trials in which IFN-$\gamma$ and/or IFN-$\beta_{ser}$ alone or in combination were administered to patients diagnosed with adenocarcinoma and nonadenocarcinoma malignancies. The statistical analysis of whether interferon administration resulted in a significant increase in the serum levels of either TAG-72 or CEA was based upon a previously published report which concluded that 95% of all serum CEA determinations taken from adenocarcinoma patients diagnosed with stable disease will fall within 36% of the initial baseline value during a 30-day period (32). The quantitative changes of CEA and TAG-72 serum levels in patients with stable disease are the result of dynamic events which include antigen clearance, the intrinsic ability of the lesion to alter its antigen phenotype, etc., which all contribute to the presence of different levels of tumor antigen in the circulating blood. Using this basis for analysis, interferon treatment increased the release of TAG-72 and CEA from adenocarcinoma lesion(s) so that a >36% rise in the serum concentration of either antigen was observed in approximately 65% of the patients. One could argue that those increases in serum TAG-72 or CEA levels were really a result of subclinical tumor progression and not due to increased tumor antigen secretion induced by the interferons. While we can offer no direct evidence to refute this assertion, several observations suggest that the interferons, indeed, were responsible for increasing TAG-72 and CEA secretion by the tumor cells. For example, our previous studies have shown that interferon treatment of human carcinoma cells *in vitro* can significantly increase CEA and TAG-72 secretion within 24–72 h (24).[4] As listed in "Materials and Methods," most serum samples from the 13 different protocols were collected within 4 days following the initiation of interferon treatment. Within that time frame, little change probably occurred in the disease status of the patients which would argue against the observed changes occurring due to a subclinical change in the tumor lesion. Second, one of the requirements for interferon up-regulation of either CEA or TAG-72 expression seems to be the presence of constitutive antigen expression. Interferon treatment does not induce *de novo* the expression of either tumor antigen by normal cells or nonexpressing malignant cells (22, 24, 25). A comparison of the sera data from patients diagnosed with nonadenocarcinoma malignancies revealed neither an increase of measurable serum levels nor the appearance of positive TAG-72 or CEA serum titers in interferon-treated patients. Thus, it appears that interferon treatment does not induce tumor antigen secretion by normal tissues. In contrast, interferon administration to patients diagnosed with adenocarcinoma whose serum TAG-72 and CEA levels were below the respective cutoff values resulted in the appearance of positive serum levels for either tumor antigen in a number of cases. For example, 7 of 32 (21.9%) patients diagnosed with adenocarcinoma whose sera samples were initially negative for TAG-72 had positive serum levels following interferon treatment. Likewise, serum CEA levels >5.0 ng/ml were measured in 2 of 15 (13.3%) patients who had serum CEA levels <5.0 ng/ml prior to interferon treatment. Both these observations suggest that the administration of interferons to patients diagnosed with gastrointestinal, lung, breast, and other adenocarcinomas increases the release of either or both tumor antigens into the circulating serum. Interestingly, interferon

treatment did not increase the serum levels or the appearance of TAG-72 or CEA in patients diagnosed with nonadenocarcinomas. While it is tempting to suggest a difference in the inducibility of tumor-associated antigen expression that would be manifested in increased antigen serum levels in the patients with adenocarcinomas *versus* nonadenocarcinomas, only additional study will determine whether such a difference exists.

The patients were grouped according to the type of interferon administered (IFN-$\gamma$ or IFN-$\beta_{ser}$ alone or in combination) as well as the site of the adenocarcinoma (*e.g.*, gastrointestinal, lung). The results suggest that both IFN-$\gamma$ and IFN-$\beta_{ser}$ alone or in combination can increase the release of TAG-72 and CEA into the sera of cancer patients The numbers of patients within the IFN-$\beta_{ser}$ and the IFN-$\beta_{ser}$ plus IFN-$\gamma$-treated groups as well as those with various sites of adenocarcinoma were too small and the dose schedules were too varied to compare relative potencies of the interferons or to determine whether a selective tumor site(s) exists from which serum tumor antigen is released as a result of interferon treatment. Subsequent retrospective trials need to be undertaken to address some of these questions.

The results of this and other studies suggest that the administration of potentially therapeutic doses of interferon to patients diagnosed with adenocarcinoma induces a measurable biological effect at the tumor site as evidenced by the increase in the release of selective human tumor markers into the circulation. The observed changes in serum tumor antigen levels stimulate considerable interest in designing possible clinical approaches that might exploit this phenomenon. The apparent ability of interferon to enhance the secretion of both TAG-72 and CEA could be of particular importance since recent data suggest that their presence in the sera of patients diagnosed with gastrointestinal adenocarcinoma may be complementary (21) and that the ability to increase either marker may facilitate the diagnosis of recurrent disease. On the other hand, only circumstantial evidence is available on the effects of serum tumor antigen on MAb targeting of human carcinoma lesions. It is believed that circulating tumor antigen levels will reduce the availability and, therefore, the uptake of the MAb at the tumor site. However, in a previous study (36), circulating TAG-72 levels actually correlated with the ability to detect colorectal carcinoma lesions by gamma scanning. While the present study must be regarded as only an observation acquired from analyzing sera samples in a retrospective manner, we believe the results provide the basis for a prospective dose escalation study of whether incorporation of an interferon schedule might be an important adjuvant in the diagnosis of recurrent, or possibly primary, adenocarcinoma as well as its effect on MAb localization of carcinoma lesions.

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