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Measurement of the Soluble Membrane Receptors for Tumor Necrosis Factor and Lymphotoxin in the Sera of Patients with Gynecologic Malignancy

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The shed portion of the 55 and 75 kDa membrane receptors
for tumor necrosis factor (TNF) and lymphotoxin (LT) have
been described in the serum of patients with cancer. This study
was designed to determine whether serum levels of the 55 and
75 kDa soluble TNF/LT receptors (sTNFr) had clinical signifi-
cance in patients with gynecologic malignancies. Serum samples
from 79 patients with ovarian, endometrial, or cervical cancer
were assayed for CA 125 levels by RIA and the 55 and 75 kDa
sTNFr levels by ELISA. Receptor and CA 125 levels were also
analyzed with respect to disease status and response to treat-
ment in banked serum samples from 14 patients with epithelial
ovarian cancer who had been followed clinically for 1–3 years.
Patient results were compared to serum samples tested from
normal donors. We found that serum levels of both sTNFr's
were elevated in the 79 patients with various gynecologic malig-
nancies [55 kDa of 3.07 ± 3.79 ng/ml ($P < 0.02$) and 75 kDa
of 2.93 ± 1.27 ng/ml ($P < 0.001$)] compared to 16 normal con-
trols (55 kDa of 0.65 ± 0.22 ng/ml and 75 kDa of 1.62 ± 0.37
ng/ml). Serum levels of 55 and 75 kDa TNF/LT receptors were
a more sensitive indicator of active cancer and had greater pre-
dictive value for detecting tumor in patients with ovarian cancer
than CA 125. The sTNFr's were also more sensitive than CA
125 in detecting persistent or recurrent tumor and measuring re-
sponse to therapy. These preliminary results suggest that mea-
surement of serum levels of 55 and 75 kDa sTNFr's, even
though not tumor specific, may be a uniquely new method for
identifying and monitoring patients with gynecologic malig-
nancy. © 1993 Academic Press, Inc.

INTRODUCTION

The host immune response to malignancy is complex
and only partially understood. Ideally, the interaction

between the tumor and host lymphoid system stimulates
a cascade of events that leads to the recognition and
ultimate destruction of the tumor by cell-mediated mech-
anisms. Destruction of the tumor results either directly
from the anti-tumor activity of cytokines [1,2] or indirectly
from cytokine activation of other host anti-tumor effector
mechanisms [3–5]. Tumor necrosis factor (TNF), which
is produced primarily by macrophages and lymphocytes
[6], and lymphotoxin (LT), produced primarily by lym-
phocytes [7], are two cytokines that are physically related
[8,9] and, while sharing common actions, also display
unique biologic activities [10]. These cytokines function
in the inductive and tissue-destructive phases of host anti-
tumor mechanisms both *in vitro* [11] and *in vivo* [12].

Although TNF and LT have anti-tumor effects, factors
that block both the *in vitro* and *in vivo* cytolytic activity
of these substances have been identified in cancer patients
[13,14]. Two distinct blocking factors (BF) were identified
in the sera of patients with a variety of cancers, the first
a 28 kDa peptide [14] that is the extracellular, N-terminal
domain of the 50 to 55 kDa cell membrane TNF–LT
receptor [15], and the second, a 28 to 30 kDa peptide
which is the extracellular domain of the 68 to 75 kDa
TNF/LT cell membrane receptor [16]. In addition, BF
bioactivity for TNF–LT has been identified in the ma-
lignant ascites of patients with ovarian cancer, but not
in ascites of patients with benign liver disease [17]. Fur-
thermore, cell-free ascites and culture supernatants from
short-term primary cultures of tumor and ascites cells
from women with different gynecologic malignancies
demonstrated BF bioactivity and also contained the sol-
uble portions of both the 55 and 75 kDa TNF/LT re-
ceptors (sTNFr) (unpublished data). These latter studies
suggest tumor tissues may be a source of BF activity in

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these patients and might explain the failure of immune mechanisms to destroy incipient gynecologic cancers.

Of potential clinical relevance is whether quantitative measures of sTNFr might provide an objective measure of response to treatment or assessment of disease status in women with gynecologic cancers. We have attempted to determine whether there are significant differences in serum levels of 55 and 75 kDa sTNFr between normal controls and women with active or inactive gynecologic cancer. In order to assess this possibility, we have measured sTNFr levels in the sera of women with gynecologic malignancy and compared it to CA 125 measurements in these same patients with respect to disease status and response to therapy. CA 125 was chosen for comparison since it is the most frequently used and most reliable marker of tumor status and response to therapy in ovarian cancer patients [18–25]. All of these studies were conducted retrospectively from a bank of serum samples collected over a 5-year period.

MATERIALS AND METHODS

Sample Collection and Patient Characteristics

The Division of Gynecologic Oncology at the University of California Irvine Medical Center has maintained a serum bank since 1985 on patients with gynecologic malignancies who had blood drawn for CA 125 levels. These were stored at $-20°C$ until being used for study. The first set of serum samples studied was collected from a 9-month period in 1991, and included 53 patients with active or history of treated ovarian cancer, 13 patients with active or a history of treated endometrial cancer, and 13 patients with active or a history of treated cervical cancer. Patients were considered without evidence of disease if they had no clinical evidence of malignancy based on exam, radiographic evaluation, or biopsy. Control samples were taken from a population of 16 normal volunteers (8 women and 8 men) who had no history of cancer and ranged in age from 19–54 years (mean 31). The second series of samples were taken from 13 patients who had been treated or were under treatment for primary epithelial ovarian cancer. There were serial serum samples stored on these patients from CA 125 evaluations during the course of their treatment and/or follow-up.

Assay for CA 125

The CA 125 levels were determined using the immunoradiometric assay of Centocor (Malvern, PA), which identifies the CA 125 antigen using a monoclonal antibody (OC 125), produced from a ovarian cell line, OVCA 433, as described by Bast et al. [18].

ELISA for TNF/LT Receptors

Antibodies for the 55- and 75-kDa sTNFr were generated in New Zealand white female rabbits according to the technique of Yamamoto et al. [26]. Recombinant 55 and 75 kDa sTNFr [27] used to generate antibodies were kindly supplied by Synergen, Inc. (Boulder, CO). The specificies of these antisera were confirmed by the establishment of an enzyme-linked immunosorbent assay (ELISA) for each receptor as described previously. For the antibodies utilized in ELISA assays, the 55 and 75 kDa sTNFr are antigenically distinct. No cross-reactivity was observed when antisera were tested against each TNF receptor, and no reactivity was observed when antisera were tested against human recombinant forms of lymphotoxin, tumor necrosis factor, interferon-γ interleukin 1, interleukin 2, interleukin 4, and interleukin 6. Briefly, the procedure for the TNF receptor ELISA was performed as follows. IgG antibody to each of the 55 and 75 kDa sTNFr was isolated from whole rabbit sera using Protein G affinity column [28]. First, 0.5 $\mu g/100$ μl of the IgG to either the 55 or 75 kDa sTNFr was bound to an ELISA plate (Corning, Corning, NY) overnight at 4°C. Subsequently, 100 μl of various concentrations of either the recombinant TNF receptor of 100 μl of serum sample was added to these wells overnight at 4°C and then washed three times with 0.2% Tween 20 in phosphate-buffered saline (PBS). Horseradish peroxidase coupling was accomplished by the methods of Nakane and Kawaoi [29], and Tijssen and Kurstak [30]. Horseradish peroxidase coupled anti-TNF receptor IgG to either the 55 or 75 kDa sTNFr was added to each well and incubated for 1 hr at 37°C. After this incubation period, the wells were washed three times with 0.2% Tween 20/PBS. The plates were then incubated with 100 μl/well of the substrate, Immunopure ABTS tablets [2,21'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt] (Pierce, Rockford, IL) in 0.1 M sodium acetate buffer, pH 4.2, for 20 min at room temperature. The amount of 55 or 75 kDa sTNFr in the unknown sample was determined by comparing it to a standard curve for the recombinant 55 or 75-kDa sTNFr by reading at 405 nm in an EAR AT ELISA plate reader (SLT-Lab Instruments, Salzburg, Austria).

Statistical Analysis

Data were analyzed using the Student t test. A P value <0.05 was considered to be significant.

RESULTS

1. Determination of Serum Levels for the 55 and 75 kDa sTNFr by ELISA in Patients with Various Gynecologic Cancers and Normal Individuals

Serum samples were collected from 16 normal individuals and from 26 patients with all stages of active ovarian, uterine, or cervical cancer. Samples were also
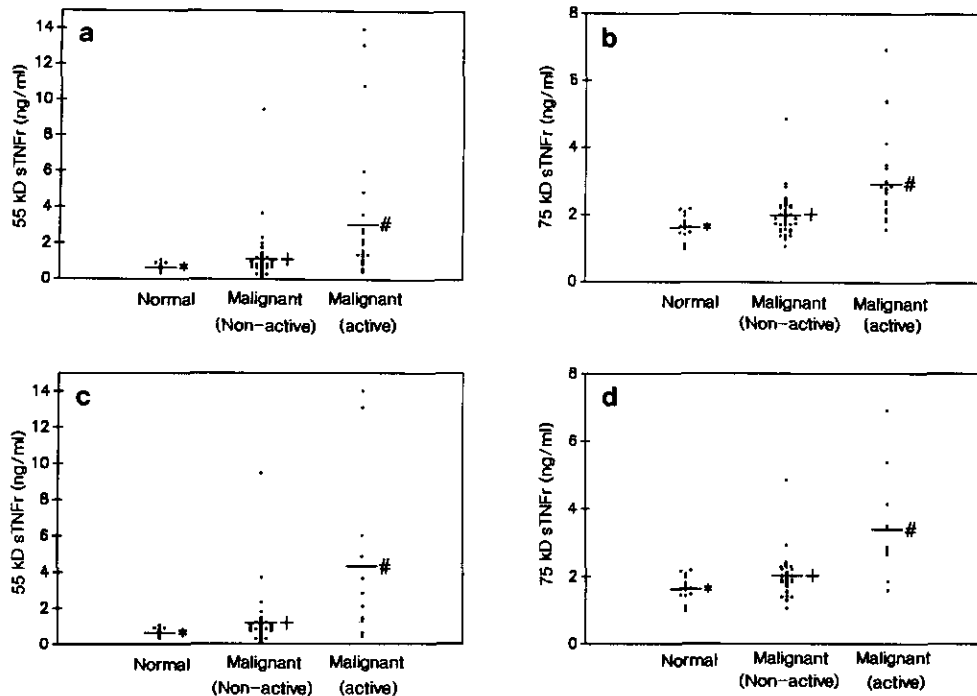
FIG. 1. Serum levels of the 55 and 75 kDa sTNFr expressed in ng/ml in normal individuals, patients with treated, nonactive (no clinical evidence of disease), and active cancer. (a) Comparison of the mean serum levels of the 55 kDa sTNFr between normal individuals * (0.65 ± 0.22 ng/ml) and patients with nonactive ovarian, endometrial, or cervical cancer $^+$ [1.12 ± 1.3 ng/ml (ns)] and patients with active ovarian, endometrial, or cervical cancer * [3.07 ± 3.79 ng/ml (P < 0.02)]. The difference between nonactive and active groups was significant at P < 0.01. (b) Comparison of the mean serum levels of the 75 kDa sTNFr between normal individuals * (1.62 ± 0.37 ng/ml) and patients with nonactive ovarian, endometrial, or cervical cancer $^+$ [1.99 ± 0.55 ng/ml (P < 0.02)] and active ovarian, endometrial, or cervical cancer * [2.93 ± 1.27 ng/ml (P < 0.001)]. The difference between nonactive and active groups was significant at P < 0.001. (c) Comparison of the mean serum levels of the 55 kDa sTNFr between normal individuals * (0.65 ± 0.22 ng/ml) and patients with nonactive ovarian cancer $^+$ [1.17 ± 1.47 ng/ml (ns)] and patients with active ovarian cancer * [4.31 ± 4.61 ng/ml (P < 0.01)]. Difference between nonactive and active groups was significant at P < 0.01. (d) Comparison of mean serum levels of the 75 kDa sTNFr between normal individuals * (1.62 ± 0.37 ng/ml) and patients with nonactive ovarian cancer $^+$ [2.02 ± 1.49 ng/ml (P < 0.001)], and normal individuals and patients with active ovarian cancer * [3.39 ± 1.49 ng/ml (P < 0.001)]. Difference between nonactive and active groups was significant at P < 0.001.

collected from women with these same cancers that had been treated, but were without clinical evidence of disease. All samples were tested in duplicate for each receptor by ELISA techniques as described under Materials and Methods. In the normal controls, the average values for the 55 and 75 kDa sTNFr were 0.65 ± 0.22 and 1.62 ± 0.37 ng/ml, respectively (Figs. 1a and 1b). These levels were not significantly different between sexes (8 women and 8 men). As shown in Fig. 1a, when compared to normal individuals, mean serum levels for the 55 kDa sTNFr in all treated patients currently without active disease were 1.12 ± 1.3 ng/ml (ns) and in all those patients with active cancer were 3.07 ± 3.79 ng/ml (P < 0.02). The difference in mean serum levels of the 55 kDa sTNFr in patients without clinical evidence of disease and those with active cancer was significant at P < 0.01. As shown in Fig. 1b, mean serum levels for the 75 kDa sTNFr were elevated in all patients without direct evidence of active cancer, 1.99 ± 0.55 ng/ml (P < 0.02) and in those with

active cancer, 2.93 ± 1.27 ng/ml (P < 0.001). The difference in mean serum levels of the 75 kDa sTNFr in patients without clinical evidence of disease and those with active cancer was also significant at P < 0.001. The group of patients with cervical or endometrial cancer were evaluated separately from those with ovarian cancer. When compared to normal individuals, mean serum levels for the 55 kDa sTNFr in patients currently without active nonovarian disease were 0.96 ± 0.45 ng/ml (P < 0.05) and in all those patients with active nonovarian cancer were 2.02 ± 2.61 ng/ml (P < 0.05). When compared to normal individuals, mean serum levels for the 75 kDa sTNFr in patients currently without active nonovarian disease were 1.91 ± 0.40 ng/ml (P < 0.05) and in all those patients with active nonovarian cancer were 2.53 ± 0.92 ng/ml (P < 0.01). Again, there was a significant difference between the serum levels of 55 and 75 kDa sTNFr in normal individuals compared to those with nonovarian gynecologic malignancies.
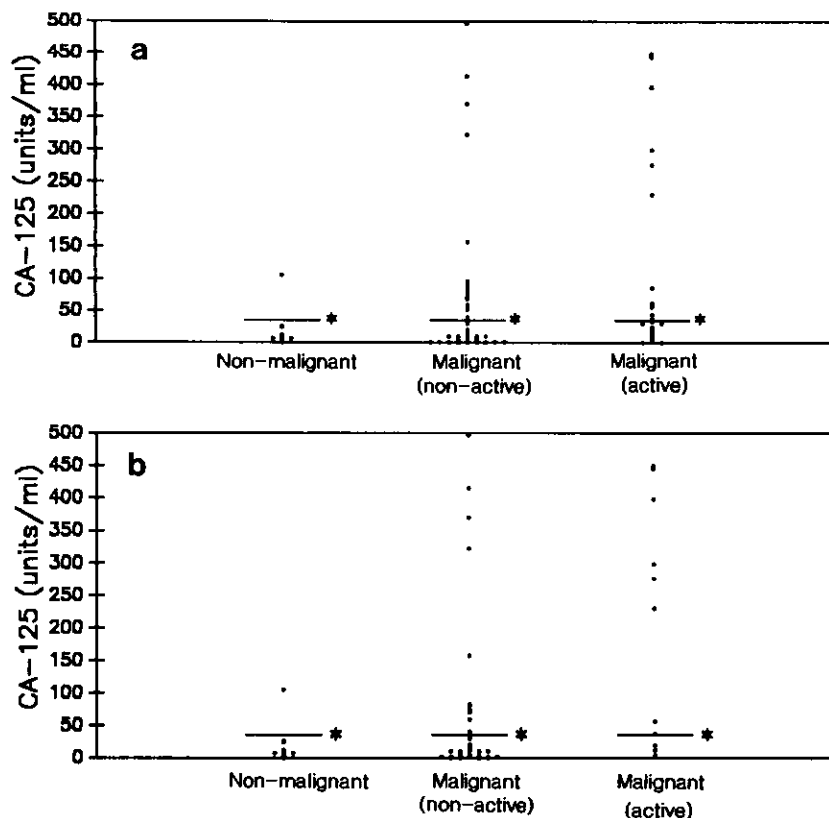
FIG. 2. Serum levels of CA 125 expressed in U/ml from the same patients shown in Fig. 1. * The upper limits of normal (35 U/ml) for CA 125 antigen in serum. (a) Serum CA 125 antigen levels in patients with ovarian, endometrial, or cervical cancer. Mean for patients without clinical evidence of disease was 53 U/ml and for patients with active cancer was 183 U/ml. Using upper limits of normal for CA 125 as 35 U/ml, 30% (16/53) of patients without evidence of active cancer had an elevated CA 125, while 50% (13/26) of patients with active cancer had an elevated CA 125. (b) Serum CA 125 antigen levels in patients with ovarian cancer. Mean for patients without clinical evidence of disease was 60 U/ml and for patients with active ovarian cancer was 365 U/ml. Using upper limits of normal for CA 125 as 35 U/ml, 29% of patients without clinical evidence of active cancer had an elevated CA 125, while 75% of patients with active ovarian cancer had an elevated CA 125.

2. Determination of the Serum Levels for the 55 and 75 kDa sTNFr by ELISA in Patients with Ovarian Cancer

From the original combined group of patients, 12 patients with active ovarian cancer and 41 patients with a history of treated ovarian cancer were evaluated as a separate group. As shown in Fig. 1c, compared to normal controls, serum levels for the 55 kDa sTNFr in patients without active ovarian cancer were 1.17 ± 1.47 ng/ml (ns), and were markedly elevated in those with active ovarian cancer, 4.31 ± 4.61 ng/ml ($P < 0.01$). The difference in mean serum levels of the 55 kDa sTNFr in patients without clinical evidence of active ovarian cancer and those with active ovarian cancer was also significant at $P < 0.001$. As shown in Fig. 1d, compared to normal controls, mean serum levels for the 75 kDa sTNFr were found to be elevated in patients without clinical evidence of active ovarian cancer, 2.02 ± 0.59 ng/ml ($P < 0.02$), and those with active ovarian cancer, 3.39 ± 1.49 ng/ml

($P < 0.001$). The difference in mean serum levels of the 75 kDa sTNFr in patients without clinical evidence of active ovarian cancer and those with active ovarian cancer was significant at $P < 0.001$.

3. Comparison of Serum Levels of 55 and 75 kDa sTNFr Receptor Levels to Serum Levels of the CA 125 Antigen

The range of CA 125 levels for all the patient samples are shown in Fig. 2a in relation to the cutoff for the upper limits of normal for CA 125 (35 U/ml) [20]. The mean for patients without clinical evidence of their gynecologic cancer was 53 U/ml (range 0–490) versus 183 U/ml (range 0–2154) in those patients with active cancer of the ovary, uterus, or cervix. In the subgroup of patients with ovarian cancer, the mean for patients without clinical evidence of their ovarian cancer was 60 U/ml (range 0–490) and 365 U/ml (range 12–2154) in those patients with active ovarian cancer as shown in Fig. 2b. The sen-

TABLE 1a

Comparison of Serum Levels of CA 125 Antigen, 55 kDa sTNFr, and 75 kDa sTNFr between Patients with Active Cancer of the Ovary, Uterus, or Cervix and Patients without Clinical Evidence of Active Cancer

	CA 125 (%)	55 kDa sTNFr (%)	75 kDa sTNFr (%)
Sensitivity	50	65	58
Specificity	70	74	92
+ Pred. value	45	55	79
− Pred. value	74	81	82

sitivity, specificity, and positive and negative predictive values were calculated for the CA 125, 55 kDa sTNFr, and 75 kDa sTNFr as to the ability of these tests to distinguish between those patients with active cancer and those without clinical evidence of their cancer. The data for all three groups analyzed together (ovary + uterus + cervix) are presented in Table 1a. The sensitivity of the CA 125 was 50%, specificity 70%, positive predictive value 45%, and negative predictive value 74%. Using 98% confidence intervals, the upper limits for our normal control 55 kDa sTNFr is 1.15 ng/ml and for the 75 kDa sTNFr is 2.48 ng/ml. These upper limits were used as the cutoff for normal in calculating the sensitivity, specificity, positive predictive value, and negative predictive value for the sTNFr's. For the same groups, the 55 kDa sTNFr sensitivity was 65%, specificity 74%, positive predictive value 55%, and negative predictive value 81%. And, for the 75 kDa sTNFr, sensitivity was 58%, specificity 92%, positive predictive value 79%, and negative predictive value 82%. When the ovarian group alone was analyzed (Table 1b), the sensitivity of the CA 125 was 75%, specificity 71%, positive predictive value 43%, and negative predictive value 91%. For the 55 kDa sTNFr, sensitivity was 83%, specificity 71%, positive predictive value 45%, and negative predictive value 94%. And, for the 75 kDa sTNFr, sensitivity was 77%, specificity 95%, positive predictive value 83%, and negative predictive value 95%.

4. Changes in the Levels of 55 and 75 kDa sTNFr and CA 125 Antigen in Sequential Serum Samples from Women Undergoing Treatment for Ovarian Cancer

Patients in this study have been followed in the gynecologic oncology clinic over months to years during treatment and follow-up for their ovarian cancer. Serum from samples collected during this time were assayed for CA 125 and stored at −20°C. Patient samples were selected for these studies and serum was tested for the 55 and 75 kDa sTNFr as described under Materials and

Methods. Serum samples from patients with four distinct patterns of change in the CA 125 over time were selected for comparison to the sTNFr levels. A representative pattern of serum-sTNFr and CA 125 levels is presented for each group. The upper limits of normal of 1.15 ng/ml for the 55 kDa sTNFr and 2.48 ng/ml for the 75 kDa sTNFr were also used in evaluating these groups of patients.

The patient representative of Group 1, patients with persistently negative CA 125, i.e., <35 U/ml, but with documented tumor at one point in time (Fig. 3A), underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, and tumor debulking for a Stage III, Grade II endometrioid ovarian carcinoma. In the first serum sample, drawn 1 month after her surgery, the CA 125 was 24 U/ml, and the 55 and 75 kDa sTNFr were 1.48 and 4.08 ng/ml, respectively. The patient completed a standard course of cis-platinum and cytoxan chemotherapy and prior to her second-look laparotomy (Fig. 3A) had a CA 125 of 6 U/ml, and 55 and 75 kDa sTNFr of 1.36 and 3.83 ng/ml, respectively. At second-look laparotomy there was persistent disease, and the patient was treated with intraperitoneal interferon-α and cis-platinum. Both sTNFr levels declined slowly after second-look surgery and intraperitoneal chemotherapy. The patient had a negative third-look laparotomy about 1 year later. The 75 kDa sTNFr level continues to slowly decline and the 55 kDa sTNFr remains stable, but both remain above the range for normals (55 kDa sTNFr at 2.03 and 75 kDa sTNFr at 4.53 ng/ml, respectively). This patient clinically remains without evidence of disease 3 years after her primary surgery.

The patient representative of Group 2, patients with declining CA 125 levels and no evidence of disease at most recent follow-up visit (Fig. 3B), underwent suboptimal surgical tumor debulking of a Stage III, Grade II papillary adenocarcinoma of the ovary. Her serum CA 125 level prior to surgery was 1540 U/ml. The level of the 75 kDa sTNFr was over four times above normal (8.44 ng/ml) while the 55 kDa sTNFr was over three

TABLE 1b

Comparison of Serum Levels for CA 125 Antigen, 55 kDa sTNFr, and 75 kDa sTNFr between Patients with Active Cancer of the Ovary and Patients Treated for Cancer of the Ovary, but Have No Clinical Evidence of Disease

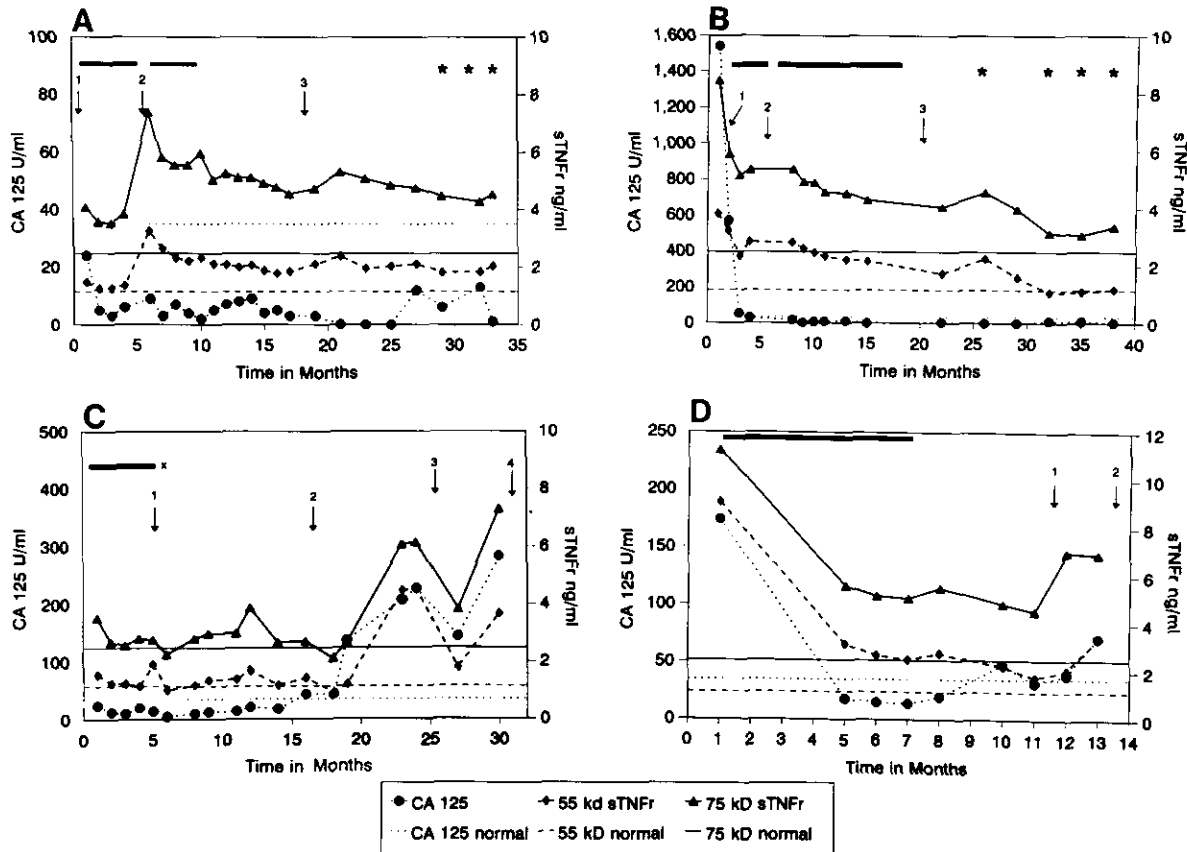|	CA 125 (%)	55 kDa sTNFr (%)	75 kDa sTNFr (%)
Sensitivity	75	83	77
Specificity	71	71	95
+ Pred. value	43	45	83
− Pred. value	91	94	95

FIG. 3. Comparison of changes in the serum levels of the 55 and 75 kDa sTNFr over time with CA 125 antigen in different patients with primary ovarian cancer. (A) Patient with Stage III endometrioid ovarian carcinoma. First serum sample collected 1 month after primary debulking surgery. 1, Tumor debulking; 2, second-look laparotomy with macroscopic disease present; 3, third-look laparotomy with no clinical evidence of cancer; solid bars, chemotherapy; * patients clinically without evidence of cancer. (B) Patient with Stage III papillary adenocarcinoma of the ovary. First serum sample collected before primary surgery. 1, Tumor debulking; 2, second-look laparotomy with macroscopic tumor present; 3, third-look laparotomy with no evidence of tumor; solid bars, chemotherapy; * patient clinically without evidence of cancer. (C) Patient with a history of recurrent Stage IC endometrioid adenocarcinoma of the ovary. At the time of entry, she was completing a course of chemotherapy (solid bar). 1, Vaginal recurrence; X, radiation implants; 2, liver metastases (biopsy-proven); 3, lung metastases; 4, patient expired. (D) Patient with Stage III endometrioid adenocarcinoma of the ovary. First serum sample collected after tumor debulking and three courses of chemotherapy. Solid bar, chemotherapy; 1, positive second-look laparotomy; 2, lung and liver metastases.

times above normal (3.80 ng/ml). The second serum sample was drawn 1 month after primary surgery and about 2 weeks after the first course of cis-platinum/cytoxan chemotherapy was given. The CA 125 level fell dramatically (570 U/ml), while the sTNFr levels also declined, but at a slower pace (55 kDa sTNFr at 3.21 and 75 kDa sTNFr at 5.90 ng/ml). At the completion of four courses of chemotherapy, her CA 125 was slightly elevated at 53 U/ml. Both sTNFr remained elevated (55 kDa sTNFr at 2.33 and 75 kDa sTNFr at 5.14 ng/ml). One month perior to second-look surgery, her CA 125 had normalized (29 U/ml), while the 55 and 75 kDa sTNFr remained elevated at 2.82 and 5.34 ng/ml. As shown in Fig. 3B, at second-look laparotomy (4 months after her primary surgery) she was found to have microscopic disease. She subsequently was treated with in-

traperitoneal interferon-α and cis-platinum. During the course of ip chemotherapy her CA 125 remained below 35 U/ml, and the receptor levels remained elevated but continued to slowly decline. A third-look laparotomy was performed about 1½ years after her first surgery and revealed no evidence of disease. The patient has had regular follow-up and remains clinically without evidence of disease and with normal CA 125 levels. Her receptor levels continue to slowly decline, with the 55 kDa sTNFr level normalizing (1.17) and the 75 kDa sTNFr level remaining elevated (3.36 ng/ml) about 3 years after her primary surgery.

The patient representative of Group 3, patients with increasing levels of CA 125 and disease recurrence (Fig. 3C), was initially diagnosed and treated 4 years prior to the collection of the serum samples in our study for a

Stage Ic, unknown grade endometrioid carcinoma of the ovary with a TAH/BSO. Postoperatively she was treated with melphalan for 9 months. She had two vaginal recurrences, the first of which was treated with resection and the second with radiation and hormonal therapy. Two months prior to the collection of serum samples at our institution, she developed another vaginal recurrence and was treated with radical vaginectomy and lymph node dissection followed by six courses of cytoxan/cis-platinum chemotherapy. Her CA 125 levels during this course of chemotherapy remained normal, but her receptor levels were slightly elevated, with the 55 kDa sTNFr near upper limits of normal. One month after the completion of the cytosan/cis-platinum chemotherapy she had yet another vaginal recurrence that was treated with radiation implants and four courses of Carboplatinum. At the time of biopsy her CA 125 was 14 U/ml and the 55 and 75 kDa sTNFr were 1.90 and 2.76 ng/ml, respectively. Her receptor levels following this treatment increased slightly and the CA 125 values remained below 35 U/ml. However, about 1 year after her third vaginal recurrence liver metastases were documented by biopsy. About 1 month after this diagnosis was made, she had a slightly elevated CA 125 of 43 U/ml. The patient refused further therapy other than hormonal. CA 125 and both the 55 and 75 kDa sTNFr levels steadily increased until she expired 6 years after her initial diagnosis. One month prior to her death the CA 125 had increased to 401 U/ml, while the 55 and 75 kDa sTNFr increased to 2.48 and 5.13 ng/ml, respectively.

The patient representative of Group 4, patients with initially decreasing CA 125 and then increasing CA 125 with disease recurrence (Fig. 3D), underwent tumor debulking at an outside institution for a Stage III, Grade I/III endometrioid adenocarcinoma of the ovary. At the time of the first serum sample she had received three courses of cytoxan/cis-platinum. Her first CA 125 in this study was 174 U/ml. At that time both of her receptor levels were elevated (55 kDa sTNFr at 9.07 ng/ml and 75 kDa sTNFr at 11.25 ng/ml). Although her CA 125 had normalized by the next sample draw, both receptor levels remained elevated (55 kDa sTNFr at 3.14 and 75 kDa sTNFr at 5.58 ng/ml). A second-look laparotomy was performed 4 months after completion of chemotherapy. Prior to that procedure the CA 125 was 62 U/ml and the 55 and 75 kDa sTNFr were 1.77 and 4.53 ng/ml. Macroscopic disease was resected at laparotomy. Following second-look laparotomy, the receptor levels increased, followed 3 months later by rapid increase in CA 125 levels. The patient was treated with radiotherapy, but within 2 months of completing radiotherapy was found to have lung and liver metastases. She died 2 months later.

DISCUSSION

The present study was conducted to determine if serum levels of 55 and 75 kDa TNF- and LT-soluble receptors may have clinical relevance. Traditionally, tumor-specific antigens have been employed as markers for serum tests for malignancy. The findings presented here suggest a possible new method of recognizing malignancy through the detection of serum levels of soluble forms of the 55 and 75 kDa TNF cell membrane receptors. Although the patient numbers are not large, these preliminary data have clearly demonstrated that the serum levels of sTNFr may have clinical value in the detection of gynecologic malignancy and in measuring response to treatment.

It appears that serum levels of these sTNFr's may be useful in distinguishing among patients with various types of active gynecologic cancer, those patients who have been treated and are clinically free of disease, and normal individuals. The data, especially for the 75 kDa sTNFr, demonstrate that the measurement of serum levels of the receptors can readily discern between patients with active disease and normal individuals. Furthermore, these results indicate that the sTNFr may be better than the CA 125 assay at distinguishing between those patients who have active cancer versus those who have been treated for but remain clinically free of disease. In patients with ovarian, cervical, or endometrial cancer, both receptors yielded better sensitivity, specificity, and positive predictive value in detection of active cancer compared to CA 125. In those patients with ovarian cancer, both receptors showed improved sensitivity, while the 75 kDa sTNFr, demonstrated definite improved specificity and positive predictive value over CA 125.

These studies also support the concept that serial monitoring of sTNFr levels in the serum of patients with ovarian cancer during treatment and follow-up more accurately reflects the disease course than CA 125 levels and may have prognostic significance in these patients. The pattern of change for sTNFr in the serum of the patients studied reflected disease status and paralleled the changes seen in CA 125 and the findings at surgery or biopsy. However, several notable differences exist between the two. First, the receptor levels fall more gradually than the CA 125 levels, which tend to drop precipitously after initiation of therapy, especially surgical debulking. Second, there are no changes in TNF receptor serum levels during and after intravenous chemotherapy. More importantly, the serum levels of both receptors remain elevated in all cases where CA 125 is negative in the presence of tumor detectable at second-look laparotomy or on biopsy of lesions found clinically or radiographically. In those patients who remain without clinical evidence of their cancer, the receptor levels continue to slowly

decline. In contrast, in patients who went on to recur and ultimately die of their disease, the receptor levels remained elevated, even at points in time where the CA 125 was negative and the patient was without clinical evidence of disease. It appears that the serum levels of sTNFr may be a more sensitive indicator of persistent or recurrent malignancy in patients undergoing therapy for ovarian cancer than serum levels of the CA 125 antigen.

In those cases where tumor recurred, the increasing CA 125 levels seemed to be a late response to heavy tumor burden, as several patients had extensive systemic metastases or rapid increase in abdominal tumor volume before expiring. CA 125 usually is elevated only when tumor volume exceeds 2 cm [22]. Therefore it is elevated in only 50% of Stage I ovarian cancers [20] and in only about 50% of positive second-look laparotomies [24,25]. Whereas detectable serum levels of the CA 125 antigen seem to depend on a certain tumor volume, it is not yet clear what affects the serum levels of soluble sTNFr. In the group of patients who were treated for ovarian cancer and remained without clinical evidence of disease, serum levels of the sTNFr slowly declined, but remained elevated. It appears that it takes a longer time for the serum levels of these receptors to normalize; however, these patients have not been followed long enough to determine whether their disease will recur.

We were the first to identify the 55 kDa sTNFr in the serum ultrafiltrates of cancer patients [13–15]. However, Aderka et al. [31], have recently reported elevated concentrations of both receptors, as measured by ELISA, in the serum of patients with colon and breast cancer. They also found that levels were higher in advanced stage disease, and that the incidence of elevation was greater with the sTNFr than with carcinoembryonic antigen [31]. Ours is the first study to look at sTNFr levels in the serum of patients with gynecologic malignancy. Although the primary source of serum used in these studies was from patients with ovarian cancer, the levels of sTNFr were also elevated in patients with endometrial or cervical cancer. CA 125 can occasionally be used as a tumor marker in endometrial or cervical cancer, but is not as sensitive as in ovarian cancer. It is possible that the sTNFr levels may be more sensitive than CA 125 in this group of patients. Further studies are needed to define the value of sTNFr serum levels in patients with endometrial or cervical carcinoma, or other malignancies of the reproductive tract.

The biologic activity and the source of the 55 and 75 kDa sTNFr in patients with malignancy is not yet clear. Factors that bind and inhibit TNF have also been isolated from the concentrated urine of normal individuals [32–34] and from patients with inflammatory and autoimmune disease [35,36]; however, no studies have been published on serum levels of the 55 and 75 kDa sTNFr in these patients. It is important to mention that the tumors from patients with gynecologic malignancy are a source of soluble TNF/LT receptors (unpublished results). Therefore it is possible to say that sTNFr's are released in the local tumor microenvironment and gain access to the systemic circulation where they can be detected in the serum. It is not clear what role(s) these receptors play in patients with malignancy. Two possible theories to explain their presence include: (a) they may have an immunoregulatory role, either directly on TNF/LT, or other cytokines and immune cells; (b) they may be released by the tumor cells as a protective mechanism against the tumor-destructive and immunoregulatory effects of TNF/LT. We have also demonstrated that ovarian cancer cells lines are capable of releasing sTNFr (unpublished results), and we are currently completing studies to help define which cells in the tumor microenvironment may be releasing these receptors. These and further studies will help clarify the significance of these receptors in patients with malignancy.

Numerous tests incorporating monoclonal antibodies for tumor antigens and other biomarkers of malignancy have been investigated in the search for a simple screening test for ovarian and other gynecologic cancers. Studies on the use of CA 125 levels in screening for ovarian cancer have shown that although it offers high specificity, the sensitivity and predictive value preclude its use as a screening test. Taken together with the cost of performing the screening test and failure of any study to demonstrate decreased morbidity or mortality as a result of CA 125 screening, CA 125 is not recommended for use as a screening tool [23]. As compared to CA 125, the levels of sTNFr's seem to be higher in patients with smaller volume disease. Although not tumor specific, it is possible that serum levels of these receptors may be more sensitive in detecting malignancy. But the more interesting possibility is that we may be able to look at cancer detection in a unique way, by actually measuring a host–tumor interaction, which is very different from measuring tumor antigen.

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REFERENCES

1. Palladino, M. A., Shalaby, M. R., Kramer, S. M., Ferraiolo, B. L., Baughman, R. A., Deleo, A. B., Crase, D., Marafino, B., Ag-

garwal, B. B., Figiari, I. S., Liggitt, D., and Patten, J. S. Characterization of the antitumor activities of human tumor necrosis factor and the comparison with other cytokines: Induction of tumor-specific immunity, *J. Immunol.* **138**, 4023–4032 (1987).

2. Bonavida, B., Tsuchitani, T., Zighelboim, J., and Berek, J. S. Synergy is documented *in vitro* with low-dose recombinant tumor necrosis factor, cisplatin, and doxorubicin in ovarian cancer cells, *Gynecol. Oncol.* **38**, 333–339 (1990).

3. Allavena, P., Peccatori, F., Maggioni, D., Erroi, A., Sironi, M., Colombo, N., Lissoni, A., Galazaka, A., Meiers, W., Mangioni, C., and Mantovani, A. Intraperitoneal recombinant γ-interferon in patients with recurrent ascitic ovarian carcinoma: Modulation of cytotoxicity and cytokine production in tumor-associated effectors and of major histocompatibility antigen expression in tumor cells, *Cancer Res.* **50**, 7318–7323 (1990).

4. Giovanni, M., Editta, B., Mingari, M., DeMaria, A., Sertoli, M., Badellino, C., Percivale, P., Catturich, A., Bertoglio, S., Civalleri, D., Santi, L., and Moretta, L. Phenotypic and functional characteristics of tumor-associated lymphocytes in patients with malignant ascites receiving intraperitoneal infusions of recombinant interleukin-2 (rIL-2), *Int. J. Cancer* **43**, 231–234 (1989).

5. Bezwoda, W. R., Seymour, L., and Dansey, R. Intraperitoneal recombinant interferon-α 2b for recurrent malignant ascites due to ovarian cancer, *Cancer* **64**, 1029–1033 (1989).

6. Wicher, J. T., and Evans, S. W. Cytokines in disease, *Clin. Chem.* **36**, 1269–1281 (1990).

7. Devlin, J., Klosterggard, K., Yamamoto, R., and Granger, G. A. Lymphotoxins: After fifteen years of research, *Lymphokines* **9**, 313–336 (1984).

8. Aggarwal, B. B., Kohr, W. J., Hass, P. E., Moffat, B., Spencer, S. A., Henzel, W. J., Bringman, T. S., Nedwin, G. E., Goeddel, D. V., and Harkins, R. N. Human tumor necrosis factor, *J. Biol. Chem.* **260**, 2345–2354 (1985).

9. Aggarwal, B. B., Moffat, B., and Harkins, R. N. Human lymphotoxin, *J. Biol. Chem.* **259**, 686–691 (1984).

10. Goeddel, D. V., Aggarwal, B. B., Gray, P. W., Leung, D. W., Nedwin, G. E., Palladino, M. A., Patton, J. S., Pennica, D., Shepard, H. M., Sugarman, B. J., and Wang, G. H. W. Tumor necrosis factors: Gene structure and biological activities, *Quant. Biol.* **51**, 597–609 (1989).

11. Green, L., Stern, M., Haviland, D., Mills, B., and Ware, C. Cytotoxins produced by antigen-specific and natural killer-like CTL are dissimilar to classical lymphotoxins, *J. Immunol.* **135**, 4034–4043 (1987).

12. Carswell, E. A., Old, L. J., Kassel, R. L., Green, S., Fiore, N., and Williamson, B. An endotoxin-induced serum factor that causes necrosis of tumors, *Proc. Natl. Acad. Sci.* **25**, 3666–3670 (1975).

13. Gatanaga, T., Lentz, R., Masunaka, I., Tomich, J., Jeffes, E. W. B., Baird, M., and Granger, G. A. Identification of TNF–LT blocking factor(s) in the serum and ultrafiltrates of cancer patients, *Lymphokine Res.* **9**, 225–229 (1990).

14. Gatanaga, T., Hwang, C., Kohr, W., Cappuccini, F., Lucci, J. A., Jeffes, E. W. B., Lentz, R., Tomich, J., Yamamoto, R., and Granger, G. A. Purification and characterization of an inhibitor (soluble tumor necrosis factor receptor) for the tumor necrosis factor and lymphotoxin from the serum ultrafiltrates of human cancer patients, *Proc. Natl. Acad. Sci.* **8**, 8781–8784 (1990).

15. Schall, T. J., Lewis, M., Koller, K. J., Lee, A., Rice, G. C., Wang, G. H. W., Gatanaga, T., Granger, G. A., Lentz, R., Raab, H., Kohr, W. J., and Goeddel, D. V. Molecular cloning and expression of a receptor for human tumor necrosis factor, *Cell* **61**, 361–370 (1990).

16. Heller, R., Seng, K., Onasch, M., Fischer, W., Chang, D., and

Ringold, G. Complementary DNA cloning of a receptor for tumor necrosis factor and demonstration of a shed form of the receptor, *Proc. Natl. Acad. Sci.* **87**, 6151–6155 (1990).

17. Cappucini, F., Yamamoto, R., DiSaia, P. J., Grosen, E., Gatanaga, M., Lucci, J. A., Ininns, E., Gatanaga, T., and Granger, G. A. Identification of tumor necrosis factor and lymphotoxin blocking factor(s) in ascites of patients with advanced and recurrent ovarian cancer, *Lymphokine Cytokine Res.* **10**, 225–229 (1991).

18. Bast, R. C., Feeney, M., Lazarus, H., Nadler, L., Colvin, R. B., and Knapp, R. C. Reactivity of monoclonal antibody with human ovarian carcinoma, *J. Clin. Invest.* **68**, 1331–1337 (1981).

19. Bast, R. C., Klug, T. L., St John, E., Jenison, E., Niloff, J. M., Laqarus, H., Berkowitz, R. S., Leavitt, T., Griffiths, C. T., Parker, L., Aurawski, V. R., and Knapp, R. C. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer, *N. Engl. J. Med.* **309**, 169–171 (1983).

20. Jacobs, I., and Bast, R. C. The CA 125 tumour-associated antigen: A review of the literature, *Hum. Rep.* **4**, 1–12 (1989).

21. Krebs, H., Goplerud, D., Kilpatrick, J., Myers, M., and Hunt, A. Role of Ca 125 as a tumor marker in ovarian carcinoma, *Obstet. Gynecol.* **67**, 473–477 (1986).

22. Hising, C., and Anjegard, I. Role as a tumour marker, *Br. J. Cancer* **50**, 765–776 (1984).

23. Berek, J., Knapp, R., Malkasian, G., Lavin, P., Whitney, C., Niloff, J., and Bast, R. CA 125 serum levels correlated with second-look operations among ovarian cancer patients, *Obstet. Gynecol.* **67**, 685–689 (1986).

24. Potter, M. E., Moradi, M., To, A. C. W., Hatch, K. D., and Shingleton, H. M. Value of serum 125 Ca levels: Does the result preclude second look? *Gynecol. Oncol.* **33**, 201–203 (1989).

25. Patsner, B., Orr, J. W., Mann, W. J., Taylor, P. T., Partridge, E., and Allmen, T. Does serum CA-125 level prior to second-look laparotomy for invasive ovarian adenocarcinoma predict size of residual disease? *Gynecol. Oncol.* **38**, 373–376 (1990).

26. Yamamoto, R. S., Ware, C. F., and Granger, G. A. The human LT system XI: Identification of LT and "TNF-like" forms from stimulated natural killers, specific and nonspecific cytotoxic human T cells *in vitro*, *J. Immunol.* **137**, 1878–1884 (1986).

27. Kohno, T., Brewer, M., Baker, S., Schwartz, P., King, M., Hale, K., Squire, C., Thompson, R., and Vannice, J. A second tumor necrosis factor receptor gene product can shed a naturally occurring tumor necrosis factor inhibitor, *Proc. Natl. Acad. Sci.* **87**, 8331–8335 (1990).

28. Ey, P. L., Pruwse, S. J., and Jenkin, C. R. Isolation of pure Ig G1, IgG2a, and Ig G2b immunoglobulins from mouse serum using protein A sepharose, *Biochemistry* **15**, 429–436 (1976).

29. Nakane, P. K., and Kawaoi, A. Peroxidase-labeled antibody: A new method of conjugation, *J. Histochem. Cytochem.* **16**, 1084–1091 (1974).

30. Tijssen, P., and Kurstak, E. Highly efficient and simple methods for the preparation of peroxidase and active peroxidase–antibody conjugate for enzyme immunoassays, *Anal. Biochem.* **136**, 451–457 (1984).

31. Aderka, D., Engelmann, H., Hornik, V., Skornick, Y., Levo, Y., Wallach, D., and Kushtai, G. Increased serum levels of soluble receptors for tumor necrosis factor in cancer patients, *Cancer Res* **51**, 5602–5607 (1991).

32. Peetre, C., Thysell, H., Grubb, A., and Olsson, I. A Tumor necrosis factor binding protein is present in human biological fluids, *Eur. J. Haematol.* **41**, 414–419 (1988).

33. Engelmann, H., Aderka, D., Rubinstein, M., Rotman, D., and Wallach, D. A tumor necrosis factor-binding protein purified to

homogeneity from human urine protects cells from tumor necrosis factor toxicity, *J. Biol. Chem.* **264,** 11974–11980 (1989).

34. Novick, D., Engelmann, H., Wallach, D., and Rubinstein, M. Soluble cytokine receptors are present in normal human urine, *J. Exp. Med.* **170,** 1409–1414 (1989).

35. Seckinger, P., Isaaz, S., and Dayer, J. M. A human inhibitor of tumor necrosis factor α, *J. Exp. Med.* **167,** 1511–1516 (1988).

36. Seckinger, P., Isaaz, S., and Dayer, J. M. Purification and biologic characterization of a specific tumor necrosis factor inhibitor, *J. Biol. Chem.* **264,** 11966–11978 (1989).