Acta Medica Okayama

Volume 43, *Issue* 4 1989 *Article* 3

AUGUST 1989

Antitumor effect of natural human tumor necrosis factor-alpha and natural human interferon-alpha in combination against human cancer transplanted into nude mice.

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Abstract

We studied the in vivo antitumor effects of natural human tumor necrosis factor-alpha (nHuTNF-alpha) and natural human interferon-alpha (nHuIFN-alpha), both of which were produced by HVJ (hemagglutinating virus of Japan)-stimulated acute lymphatic B cell leukemia line, BALL-1 cells. To clarify the interaction between nHuTNF-alpha and nHuIFN-alpha, we used novel experimental models of lung metastasis and intraabdominal carcinomatosis which we developed in nude mice using a human tumor line, RPMI 4788. While the intravenous administration of nHuTNF-alpha or nHuIFN-alpha alone inhibited lung metastasis, the two cytokines given in combination synergistically inhibited lung metastasis. In a comparative study, nHuTNF-alpha and recombinant human interferon-gamma (rHuIFN-gamma) in combination also synergistically inhibited lung metastasis. Treatment with nHuTNF-alpha and nHuIFN-alpha combined significantly prolonged the survival of nude mice with intraabdominal carcinomatosis. Complete regression of five different human tumor xenografts was achieved by the simultaneous intratumoral injection of nHuTNF-alpha and nHuIFN-alpha. Histological examination revealed that tumor cell lysis occurred 24 h after the intratumoral administration of the cytokines. No significant signs of toxicity to nude mice were observed at any dose tested. The synergism of nHuTNF-alpha and nHuIFN-alpha may allow treatment at a relatively low dose range, thus minimizing side effects. The wide range of anticancer activity of these agents may provide better therapeutic efficacy. The in vivo assay systems which we have developed are useful for the analysis of the biological activities and interactions of cytokines and chemotherapeutic drugs.

KEYWORDS: tumor necrosis factor, interferon-?, human tumor, nude mouse

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*PMID: 2801185 [PubMed - indexed for MEDLINE]

Acta Med Okayama 43 (4) 211-221 (1989)

Antitumor Effect of Natural Human Tumor Necrosis Factor-α and Natural Human Interferon-α in Combination against Human Cancer Transplanted into Nude Mice

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We studied the *in vivo* antitumor effects of natural human tumor necrosis factor-α (nHuTNF-α) and natural human interferon-α (nHuIFN-α), both of which were produced by HVJ(hemagglutinating virus of Japan)-stimulated acute lymphatic B cell leukemia line, BALL-1 cells. To clarify the interaction between nHuTNF-α and nHuIFN-α, we used novel experimental models of lung metastasis and intraabdominal carcinomatosis which we developed in nude mice using a human tumor line, RPMI 4788. While the intravenous administration of nHuTNF-α or nHuIFN-α alone inhibited lung metastasis, the two cytokines given in combination synergistically inhibited lung metastasis. In a comparative study, nHuTNF-α and recombinant human interferon-γ (rHuIFN-γ) in combination also synergistically inhibited lung metastasis. Treatment with nHuTNF-α and nHuIFN-α combined significantly prolonged the survival of nude mice with intra-abdominal carcinomatosis. Complete regression of five different human tumor xenografts was achieved by the simultaneous intratumoral injection of nHuTNF-α and nHuIFN-α. Histological examination revealed that tumor cell lysis occurred 24 h after the intratumoral administration of the cytokines. No significant signs of toxicity to nude mice were observed at any dose tested. The synergism of nHuTNF-α and nHuIFN-α may allow treatment at a relatively low dose range, thus minimizing side effects. The wide range of anticancer activity of these agents may provide better therapeutic efficacy. The *in vivo* assay systems which we have developed are useful for the analysis of the biological activities and interactions of cytokines and chemotherapeutic drugs.

Key words : **tumor necrosis factor, interferon-α, human tumor, nude mouse**

Tumor necrosis factor (TNF) was discovered by Carswell *et al.* during a study of the antitumor activity of serum from mice infected with bacillus Calmette-Gue'rin (BCG) and subsequently injected with endotoxin (1). Serum from such mice contains a factor that

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Abbreviations used : TNF, tumor necrosis factor ; nHuTNF-α, natural human tumor necrosis factor-α ; nHuIFN-α, natural human interferon-α; rHuIFN-γ, recombinant human interferon-γ; IFNs, interferons ; BCG, bacillus Calmette-Gue'rin ; HVJ, hemagglutinating virus of Japan ; PMA, 4β-phorbol 12β-myristate 13-acetate.

induces hemorrhagic necrosis of certain mouse sarcomas *in vivo* and has cytotoxic/cytostatic effects on mouse and human tumor cells *in vitro*. TNF is also produced by stimulated macrophage, T cell and B cell lines *in vitro* (2-4). Nedwin *et al.* (5) have designated TNF produced by PMA(4β-phorbol 12β-myristate 13-acetate)-stimulated HL 60 cells established from a promyelocytic leukemia as TNF-α and lymphotoxin derived from RPMI 1788 cells established from normal B cells as TNF-β.

TNF-α is a multifunctional cytokine which may have important roles *in vivo*. Gifford *et al.* (6), Ziegler-Heitbrock *et al.* (7) and Zacharchuk *et al.* (8) indicated that macrophage cytolytic factor was related to or identical to TNF-α. Ortaldo *et al.* (9) showed that a soluble factor produced by murine natural cytotoxic cells (NC cells) was TNF-α. Degliantoni *et al.* (10) observed that a soluble natural killer cell cytotoxic factor was completely inactivated by the anti-TNF-α antibody. Takeda *et al.* (11) indicated that the differentiation-inducing factor (DIF) in conditioned media of mitogen-stimulated human peripheral blood leukocytes, which induce human myelogenous leukemic cells to differentiate toward monocyte and macrophage, was TNF-α. In addition, findings by Beutler *et al.* (12, 13) indicated that cachectin and TNF-α might be identical.

We have previously shown that the combination of highly purified natural human TNF-α (nHuTNF-α) and highly purified natural human interferon-α (nHuIFN-α), both produced by HVJ-stimulated B cells of an acute lymphatic leukemia line (BALL-1 cells) (14, 15), synergistically inhibited the growth of a human colon cancer cell line, RPMI 4788, *in vitro* (16). It has generally been difficult to test the interactions of human cytokines with species specificity because of the lack of experimental systems which are sensitive enough. In order to learn more about the activity of these cytokines, we tested the *in vivo* antitumor activities of nHuTNF-α and HuIFNs against RPMI 4788 cells using novel experimental models of human cancer metastases which we developed. Furthermore, we investigated the antitumor activity of these agents in combination against a spectrum of human tumors transplanted into nude mice.

Materials and Methods

Human tumor lines. RPMI 4788 cells derived from a human colon cancer were supplied by Roswell Park Memorial Institute, Buffalo, NY, USA (17). These tumor cells were maintained in RPMI 1640 medium (Nissui, Seiyaku Co., Ltd., Tokyo, Japan) supplemented with 10% fetal bovine serum. Tumor cells were harvested from subconfluent cultures by dispersing the cell layers with a solution of 0.25% trypsin in Hanks' balanced salt solution (HBSS) to use for the transplantation into nude mice. RPMI 4788 cells have a stable karyotype and express human transferrin receptors which can be recognized with OKT-9 murine monoclonal antibody (Ortho Diagnostic Systems, Inc., Westwood, USA). The histological examination of a solid tumor of RPMI 4788 cells which grew subcutaneously in CD-1 nude mice showed a poorly differentiated adenocarcinoma (18). HuH-7, a human hepatocellular carcinoma was obtained from a collection of Prof. J. Sato (Okayama University Medical School, Okayama, Japan) (19). MX-1 derived from a human breast cancer was supplied from Kyowa Hakko Co., Ltd., Tokyo, Japan (20). Other tumors, a gastric cancer line GC-1, a bowel cancer line CC-2 and a lung cancer line LC-1, which were serially transplanted in nude mice, were established and serially maintained in our laboratories (18).

Preparations of TNF and IFN. nHuTNF-α and nHuIFN-α were obtained from Hayashibara Biochemical Laboratories, Inc., Okayama, Japan. nHuTNF-α and nHuIFN-α were produced by HVJ-stimulated BALL-1 cells and purified to homogeneity as judged by SDS-polyacrylamide electrophoresis. Molecular weight of nHuTNF-α was

17,000 (21, 22). nHuIFN-α was composed of three subtypes, α-2, α-7 and α-8, according to Weissmann's notation (23, 24). The amino acid sequence of nHuTNF-α exhibited 98.7% homology with that of HuTNF-α, derived from PMA-stimulated HL 60 cells as reported previously (16). Titers of nHuTNF-α were based on a 50% reduction in cell viability compared with control values, which is calculated on the basis of the cytolytic activity of nHuTNF-α against actinomycin D-treated L 929 mouse fibroblast cells using a method developed for TNF or lymphotoxin by Eifel *et al.* (15, 16, 25). The specific activity was 10^9U/mg protein. When our standard was titrated against a Japanese reference (J-PS 5K01, National Institute of Health, Tokyo, Japan), 350U equaled 1 JRU. The titers of nHuIFN-α were determined with a cytopathic effect inhibition assay using human FL cells challenged with sindbis virus, and standardized against the international reference preparations of human IFN-α (Ga23-901-532). nHuTNF-α was diluted with saline. Inactivated serum of CD-1 nude mice was used for stabilization of nHuTNF-α. rHuIFN-γ was supplied by Kyowa Hakko Co., Ltd., Tokyo, Japan and titrated against a reference (J-Ref-02, National Institute of Health, Tokyo, Japan) (26). The specific activity was 5×10^6JRU/mg protein.

Animals. Male nude mice of CD-1 background (nu/nu) were obtained at 4 to 6 weeks of age from Charles River Japan, Inc., Atsugi, Kanagawa, Japan. Animals were kept under specific pathogen-free conditions.

Induction of lung metastasis. Mice were inoculated in the tail vein with 2.5×10^6 viable RPMI 4788 cells in 0.1 ml of inoculation solution (27). The mice were then randomly allocated to respective groups. At least 5 mice were included in each group. The mice were killed 23 days after the tumor inoculation. A 15% solution of india ink in PBS was injected into the bronchus of each mouse. The removed lungs were bleached in Fecket's solution allowing the metastases to be readily counted since they formed discrete white nodules on the surface of the lungs.

Induction of intraabdominal carcinomatosis with ascites. Mice were inoculated intraperitoneally with 5×10^6 viable RPMI 4788 cells in 0.5 ml of inoculation solution (27). The mice were randomly allocated to 4 groups of 7 mice each. In the survival experiments, mice were observed until death.

Preparation of subcutaneous tumor. Two-tenths milliliter of a tumor cell suspension containing 2×10^6 of RPMI 4788 cells was injected subcutaneously into the back of each mouse of one group (27). Solid tumors were cut with scissors into blocks 2-3 mm in each dimension, and two blocks of each tumor line were inoculated subcutaneously into the back of each mouse of another group. The tumors were allowed to grow for 2 to 3 weeks until the tumor size became about 100 mm^3. Treatment with cytokines was then started. Tumors were observed daily. The tumors were measured weekly with the aid of microcalipers, and the tumor volumes were approximated by the formula $L\times W^2/2$, where L is the length and W is the width of the tumor.

Results

Effects of nHuTNF-α and HuIFNs on experimental metastasis. The lung metastasis model was used to examine the therapeutic interactions between nHuTNF-α and HuIFNs. On day 0, the mice were inoculated intravenously with 2.5×10^6 RPMI 4788 cells, and from day 2 to day 22, the mice were given daily intravenous injections of nHuTNF-α and/or HuIFNs or saline. The mice were sacrificed on day 23. The results are summarized in Table 1. When nHuTNF-α and nHuIFN-α were administered singly, the percent inhibition of metastasis was 42.2% and 54.0%, respectively. When administered in combination, the percent inhibition was 100%, *i.e.*, no metastatic nodules were observed in any mouse (Fig. 1). The inhibitory effect of the combination of nHuTNF-α and nHuIFN-α was significantly greater than that of either agent alone. The effect of rHuIFN-γ in combination with nHuTNF-α was also tested. Again, the inhibitory effect of the agents was significantly greater when given in combination (99.3%) than when given singly (nHuTNF-α, 42.2%; rHuIFN-γ,

50.6%). The inhibitory effect of nHuTNF-α in combination with either nHuIFN-α or rHuIFN-γ against lung metastasis of RPMI 4788 cells was thus found to be more than additive. No metastatic focus was observed histologically in the lungs of

Table 1 Effect of intravenous administration of nHuTNF-α and/or HuIFNs against lung metastasis of RPMI 4788 cells transplanted to nude mice

Cytokines administered[a]	Number of metastatic nodules[b,c]	Range	% Inhibition[d]	FBW/IBW[e]
Saline	194.0 ± 32.7	(85-213)		1.22
nHuTNF-α	112.3 ± 19.6	(63-162)	42.2	1.25
nHuIFN-α	87.4 ± 10.6	(44-108)	54.0	1.31
rHuIFN-γ	95.8 ± 23.6	(50-197)	50.6	1.36
nHuTNF-α + nHuIFN-α	0 ± 0	(0-0)	100.0	1.20
nHuTNF-α + rHuIFN-γ	1.3 ± 0.6	(0-3)	99.3	1.23

a: Saline control and cytokine test animals were given intravenous injections starting on day 2 after tumor cell injection. Saline control, 0.1 ml daily for 21 days; nHuTNF-α, 1×10^6 U/mouse/day for 21 days; nHuIFN-α, 1×10^6 IU/mouse/day for 21 days; rHuIFN-γ, 5×10^5 JRU/mouse/day for 21 days; nHuTNF-α plus nHuIFN-α, administered simultaneously at 1×10^6 U and 1×10^6 IU/mouse/day, respectively, for 21 days; nHuTNF-α plus rHuIFN-γ, 1×10^6 U and 5×10^5 JRU/mouse/day, respectively, for 21 days.

b: Mean ± standard error.

c: Statistical significance of differences was examined by Student's *t*-test. (nHuTNF-α + nHuIFN-α) versus nHuTNF-α alone, $p < 0.01$ nHuIFN-α alone, $p < 0.001$. (nHuTNF-α + rHuIFN-γ) versus nHuTNF-α alone, $p < 0.001$ or rHuIFN-γ alone, $p < 0.001$.

d: $\%\ \text{Inhibition} = \left(1 - \frac{\text{Mean no of metastatic nodules of experiment}}{\text{Mean no of metastatic nodules of control}}\right) \times 100$

e: FBW/IBW, final body weight/initial body weight on day 2.

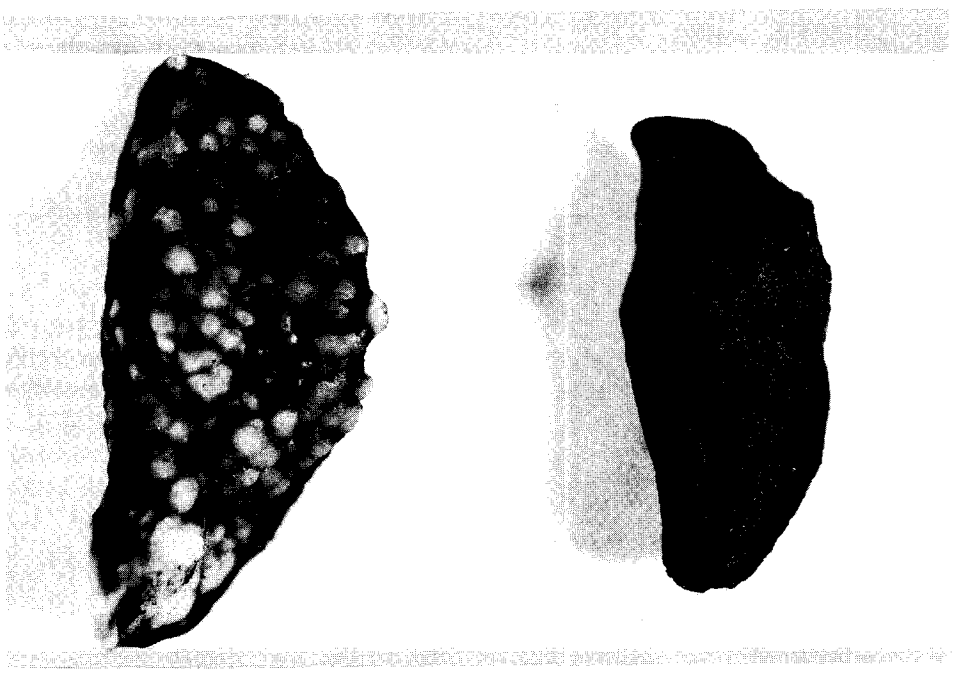

Fig. 1 Representative pulmonary lobes of mice inoculated with RPMI 4788 cells (see Materials and Methods and Table 1) and treated with saline (left) or nHuTNF-α plus nHuIFN-α (right).

mice given the combination of nHuTNF-α and nHuIFN-α. Weight loss of the mice and obvious side effects due to the treatments were not observed. Additional experiments showed that the results summarized in Table 1 were reproducible.

Effect of nHuTNF-α and nHuIFN-α on the survival of mice with intraabdominal carcinomatosis. On day 0, the mice were inoculated intraperitoneally with 5×10^6 RPMI 4788 cells, and from day 2 to day 11, the mice were given daily intraperitoneal injections of nHuTNF-α and/or nHuIFN-α or saline. In the saline control group, accumulation of ascites was observed about 3 weeks after the inoculation of RPMI 4788 cells. The mean survival of control mice was 50.4 days. The mean survival of mice which received either nHuTNF-α or nHuIFN-α was 50.3 days and 52.6 days, respectively. The mice which were given a combination of nHuTNF-α and nHuIFN-α survived significantly longer (82.7 days), than the mice which were given a single agent (Fig. 2). The cure rate (survival for over 100 days) was 57.1% in the group treated with the

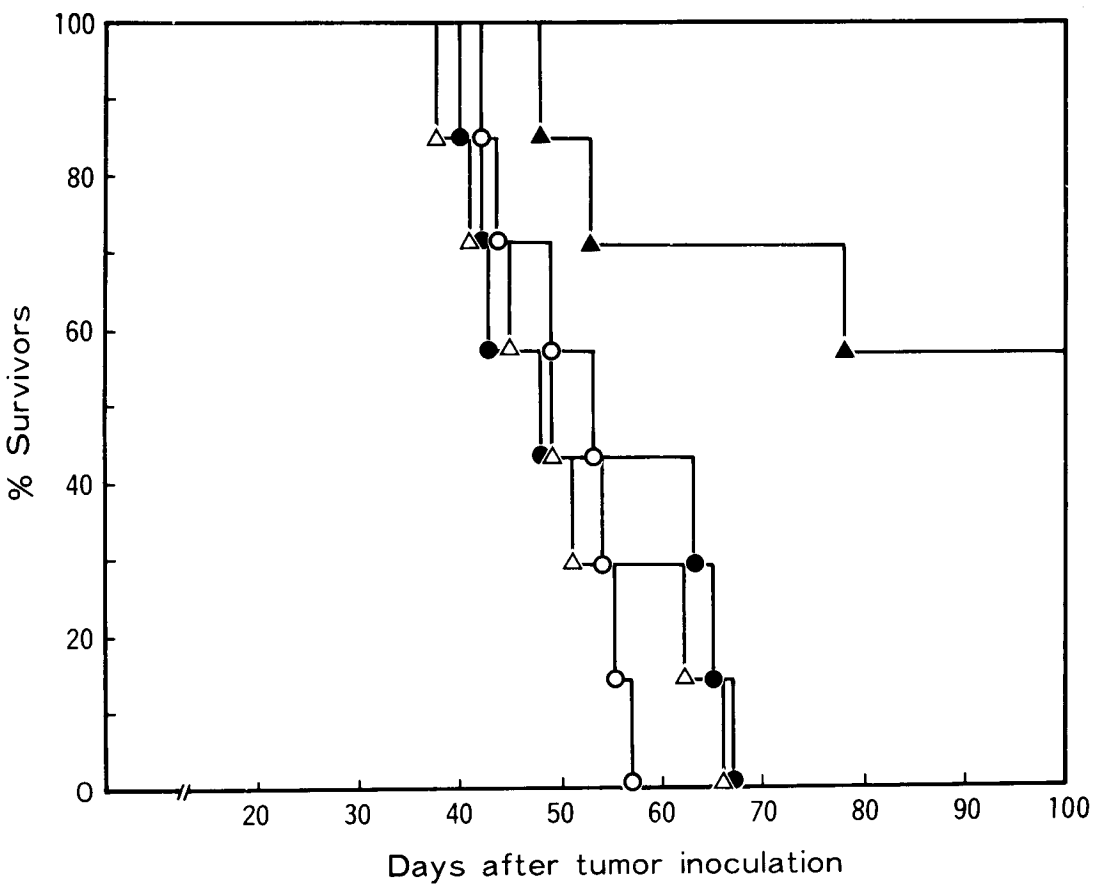

Fig. 2 Survival curves of mice inoculated intraperitoneally with RPMI 4788 cells and treated with nHuTNF-α and/or nHuIFN-α. Each group consisted of 7 mice. A) Saline (control) 0.1 ml daily for 10 days (○——○); B) nHuTNF-α, 1×10^6 U/mouse/day for 10 days (△——△); C) nHuIFN-α, 1×10^6 IU/mouse/day for 10 days (●——●); D) nHuTNF-α plus nHuIFN-α, 1×10^6U and 1×10^6 IU/mouse/day, respectively, for 10 days (▲——▲). Differences between D and A, B and C were statistically significant by Wilcoxon's test ($p < 0.05$).

combination. There was no significant difference in the mean survival time between the control group and the single treatment group. It is clear from these results that nHuTNF-α and nHuIFN-α acted in combination to extend the survival time of mice with intraabdominal carcinomatosis.

Effect of nHuTNF-α and nHuIFN-α in combination on subcutaneously inoculated RPMI 4788 cells. Fig. 3 shows the growth curve of subcutaneous inoculated RPMI 4788 cells. Tumor growth inhibition was calculated as follows: % Inhibition $= 100 \times (1 -$ mean V_{21}/V_0 ratio of experiment/mean V_{21}/V_0 ratio of control), where V_{21} is the mean tumor volume on day 21 and V_0 is the mean tumor volume on day 0. When nHuTNF-α and nHuIFN-α were administered intravenously in combination, the growth inhibition was 75.9% at a dose of 10^7U and 10^7 IU/mouse/day, respectively, 49.1% at a dose of 10^6U and 10^6 IU/mouse/day, respectively and 7.4% at a dose of 10^5U and 10^5 IU/mouse/day, respectively. Intratumoral combined administration of nHuTNF-α and nHuIFN-α, each at 10^5U and 10^5 IU/mouse/day,

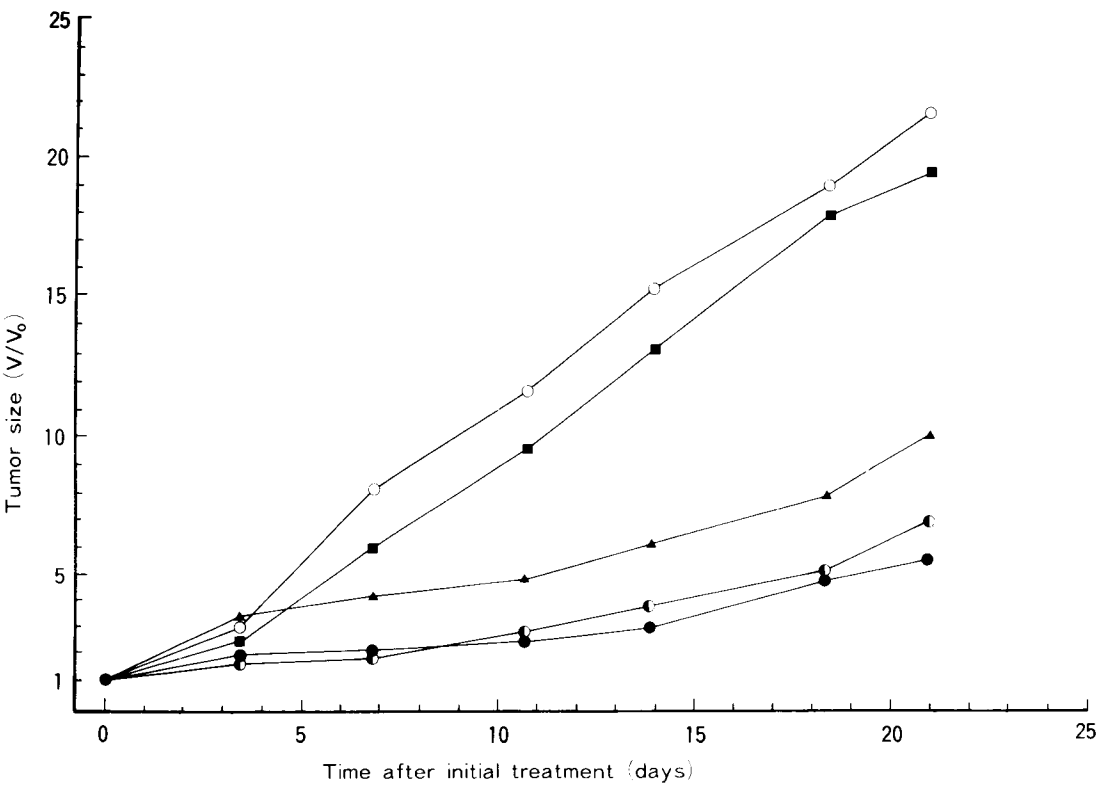

Fig. 3 Growth curves of RPMI 4788 cell tumors subcutaneously inoculated in mice treated intravenously (i.v.) or intratumorally (i.t.) with nHuTNF-α and nHuIFN-α in combination. A) Saline (control) i.v., 0.1 ml for 21 days (○——○). B) nHuTNF-α plus nHuIFN-α, 1×10^7U and 1×10^7 IU/mouse/day, respectively, i.v. for 21 days (●——●). C) nHuTNF-α plus nHuIFN-α, 10^6U and 1×10^6 IU/mouse/day, respectively, i.v. for 21 days (▲——▲). D) nHuTNF-α plus nHuIFN-α, 1×10^5U and 1×10^5 IU/mouse/day, respectively, i.v. for 21 days (■——■). E) nHuTNF-α plus nHuIFN-α, 1×10^5U and 1×10^5 IU/mouse/day, respectively, i.t. for 21 days (◐——◐). No significant difference was seen between the saline i.v. group and saline i.t. group (data not shown). Differences between A and B, C and E were statistically significant by Student's t-test ($p < 0.001$). Tumor growth is expressed as the ratio of V/V_0, where V is the mean tumor volume on the day of evaluation and V_0 is the mean tumor volume on the day of the start of the administration of the respective cytokines.

resulted in 69.9% growth inhibition. Intravenous combined administration of nHuTNF-α and nHuIFN-α, each at 10^7U and 10^7 IU/mouse/day, respectively, exhibited approximately the same degree of growth inhibition as the intratumoral administration at 10^5U and 10^5 IU/mouse/day.

Effect of nHuTNF-α and nHuIFN-α in combination on the growth of human tumor lines. Intratumoral administration of nHuTNF-α and nHuIFN-α (each at 1×10^6U and 10^6 IU/mouse/day for 7 days) resulted in complete regression of GC-1 (gastric cancer), CC-2 (bowel cancer), HuH-7 (hepatoma), MX-1 (breast cancer) and LC-1 (lung squamous cell carcinoma) (Fig. 4). The cytokines inhibited tumor growth quite effectively even at one-tenth of the above doses, when admini-

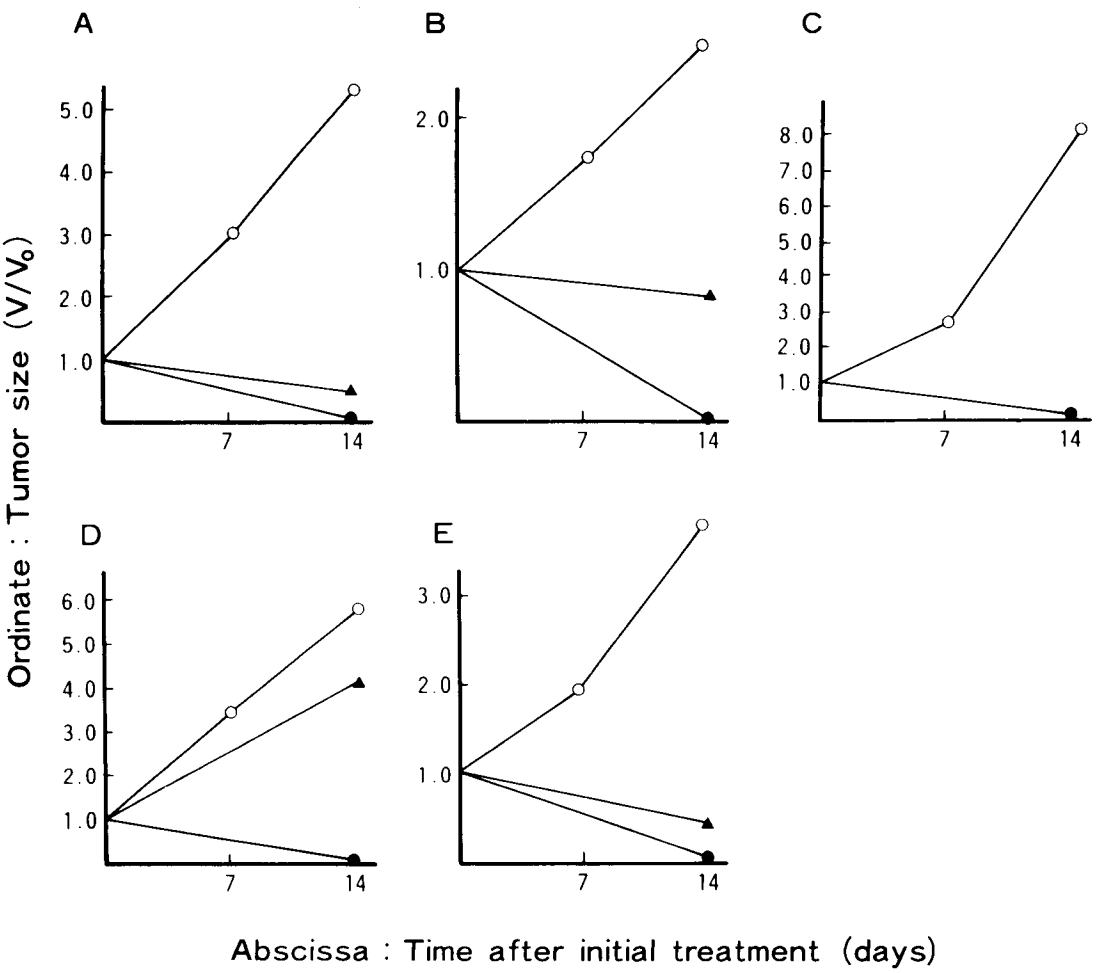

Fig. 4 Effect of intratumoral administration of nHuTNF-α and nHuIFN-α in combination on growth of xenografts of 5 human cancer cell lines in mice. (○——○), Saline (control), 0.1 ml daily for 7 days; (●——●), nHuTNF-α plus nHuIFN-α, 1×10^6U and 1×10^6IU, respectively, daily for 7 days; (▲——▲), nHuTNF-α plus nHuIFN-α, 1×10^5U and 1×10^5IU, respectively, daily for 7 days. Means of data for experimental groups of at least 5 mice are indicated. Tumor growth is expressed as the ratio of V/V_0, where V is the mean tumor volume on the day of evaluation and V_0 is the mean tumor volume on the day of the start of the administration of drugs. A, GC-1 (gastric cancer); B, CC-2 (bowel cancer); C, HuH-7(hepatoma); D, MX-1(breast cancer; E, LC-1(lung squamous cell carinoma).

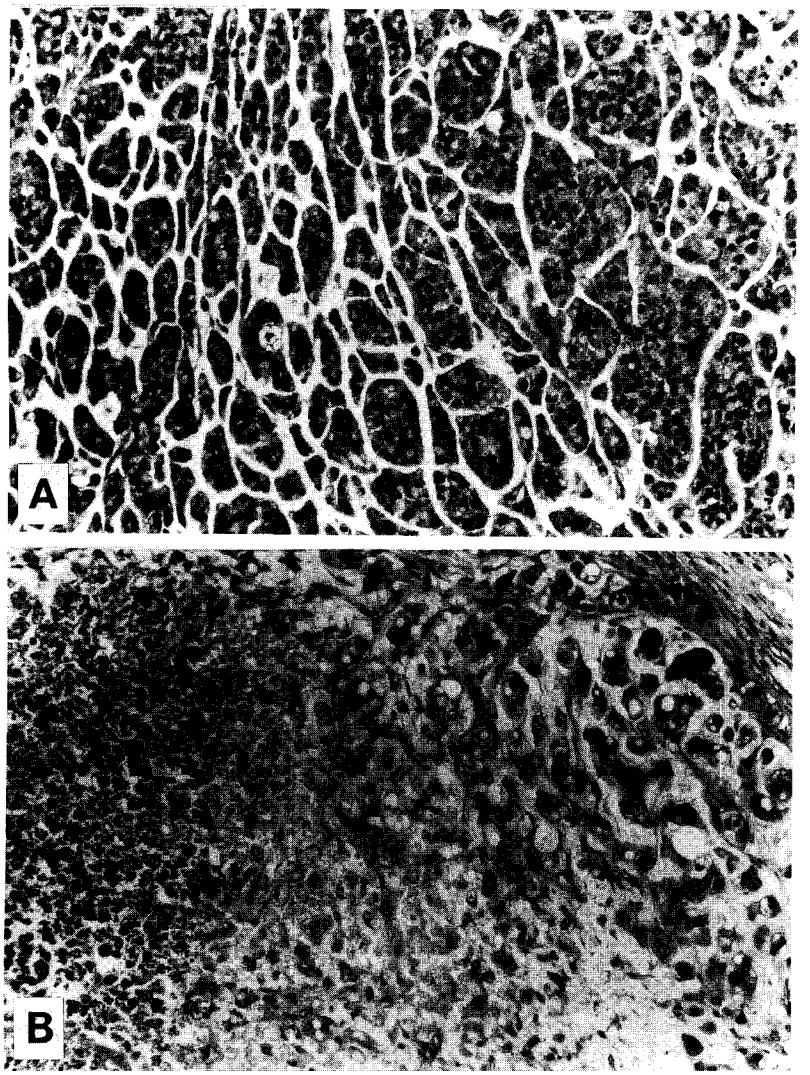

Fig. 5 Histological appearance of control (saline) (A) and [nHuTNF-α (1×10^6U) plus nHuIFN-α (1×10^6IU)]-treated CC-2 bowel cancer tumor (B). Tumors were excised 24 h after the therapy and fixed in 10% formalin. Stained with hematoxylin and eosin stain. ×100.

stered intratumorally. These observations indicate that the combination of nHuTNF-α and nHuIFN-α has a broad spectrum of anti-cancer activity. It was shown histologically that a single intratumoral injection of the cytokines at doses of 10^6U nHuTNF-α and 10^6IU nHuIFN-α/mouse caused significant cell destruction. The CC-2 bowel cancer cells showed medullary growth in the saline control (Fig. 5-A). The cytokines caused cellular ballooning, vacuolation, nuclear picnosis and cell lysis (Fig. 5-B).

Discussion

Few reports have described in detail the *in vivo* synergism between human TNF and human IFNs (28). We have developed novel experimental models of human cancer metas-

tasis using a human colon cancer cell line, RPMI 4788 cells, to investigate the *in vivo* antitumor activity of human cytokines which have species specificity (27). Using these experimental models, we demonstrated in the present study that nHuTNF-α and nHuIFN-α, as well as nHuTNF-α and rHuIFN-γ, synergistically inhibited metastasis to the lung of RPMI 4788 cells transplanted in nude mice. Treatment with nHuTNF-α and nHuIFN-α in combination significantly prolonged the survival time of nude mice with intraabdominal carcinomatosis. These cytokines in combination had potent anticancer activities against a broad spectrum of cancers. The mechanism of the synergism observed in this study is unknown. TNF binds to TNF-receptor, and cell surface binding of TNF is followed by its internalization and intracellular degradation (29). IFN-α and IFN-β both increase TNF receptors in HeLa cells, albeit with a lower efficacy than IFN-γ (30, 31). Tsujimoto *et al.* demonstrated that IFN-γ can directly stimulate the accumulation of mRNA which codes TNF receptors (32). Nevertheless, they stated that increased TNF receptor expression after incubation of cells with IFN might not be a major factor of the synergism between TNF and IFNs. This conclusion is based on their observation that treatment of cells with IFN-γ together with IFN-β prevented the enhanced binding of TNF seen in cells incubated with IFN-γ alone, whereas the addition of IFN-β did not interfere with the synergistic enhancement of TNF cytotoxicity produced by IFN-γ. The synergism between TNF and IFNs is probably due to the activation of separate inhibitory pathways whose combined cytotoxic actions are greater than the sum of their separate actions.

TNF, as well as IFNs, augments host immune systems against neoplasms (33). Haranaka *et al.* observed that the effects of partially purified TNF on Meth-A sarcoma were stronger in BALB/C nu/+ than in nu/nu mice (34). They concluded that TNF induced a host mediated factor which was attributable to the antitumor effects in addition to a direct cytotoxic antitumor effect. Peters *et al.* have suggested that natural killer (NK) cell-mediated cytotoxicity can be attributed to a combination of cytokines which include TNF, IFN-γ, interleukin 1 and other undefined cytokines (35). These reports suggest that TNF and IFN mutually act physiologically on immune systems. However, the antitumor effect of nHuTNF-α and nHuIFN-α in the present study may be direct since the NK cell activity of the nude mice used was not augmented significantly, even when the cytokines were combined, and the mononuclear cell infiltration in nude mice was found to be quite poor in tumors or target organs treated with the cytokines (data not shown).

In view of the strong anticellular activity on a broad range of cancer cells *in vitro*, it is puzzling that TNF-α has not proved very effective in clinical trials, even at high doses (36). Serious side effects including weight loss are observed upon treatment with TNF-α at a high dose because of its cachectin like activity (36). Nevertheless, in the present study, no weight loss was observed in TNF-α treated mice (Table 1). This absence of weight loss may be due to the low dose of TNF-α used in the present study. The synergism of nHuTNF-α and nHuIFN-α may allow treatment at relatively low doses thereby minimizing side effects. The wide range of anticancer activity of these agents may provide better therapeutic efficacy. In fact, nHuTNF-α and nHuIFN-α in combined treatment appeared to elicit significant effects against several kinds of human cancers, such as breast cancer, hepatoma and renal cancer, in a clinical trial (37).

Acknowledgments. The authors are grateful to J. Minowada, M.D., D.M.S., executive director, Fujisaki Cell Center, Hayashibara Biochemical Laboratories, Inc., Okayama, Japan for reviewing our manuscript. The authors also thank Hayashibara Biochemical Laboratories, Inc. for supplying TNF and IFN, Kyowa Hakko Co., Ltd., Tokyo, Japan for supplying IFN and Dr. H. Kondo for his helpful assistance.

References

1. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N and Williamson B: An endotoxin induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci USA (1975) **72**, 3666-3670.
2. Rubin BY, Anderson SL, Sullivan SA, Williamson BD, Carswell EA and Old LJ: Purification and characterization of human tumor necrosis factor from the Luk II cell line. Proc Natl Acad Sci USA (1985) **82**, 6637-6641.
3. Williamson BD, Carswell EA, Rubin BY, Prendergast JS and Old LJ: Human tumor necrosis factor produced by human B-cell lines: Synergistic cytotoxic interaction with human interferon. Proc Natl Acad Sci USA (1983) **80**, 5397-5401.
4. Creasey AA, Reynolds MT and Laird W: Cures and partial regression of murine and human tumors by recombinant human tumor necrosis factor. Cancer Res (1986) **46**, 5687-5690.
5. Nedwin GE, Naylor SL, Sakaguchi AY, Smith D, Jarrett-Nedwin J, Pennica D, Goeddel DV and Gray PW: Human lymphotoxin and tumor necrosis factor genes: Structure, homology and chromosomal localization. Nucleic Acid Res (1985) **13**, 6361-6373.
6. Gifford GE, Loewenstein J, Yamin A and Gallily R: Correlation of macrophage-mediated tumor cell lysis with the production of macrophage cytolytic factor (CF). Preliminary characterization of a factor inhibiting CF production. Int J Cancer (1986) **37**, 73-79.
7. Ziegler-Heitbrock HWL, Möller A, Linke RP, Haas JG, Rieber EP and Riethmuller G: Tumor necrosis factor as effector molecule in monocyte mediated cytotoxicity. Cancer Res (1986) **46**, 5947-5952.
8. Zacharchuk CM, Drysdale BE, Mayer MM and Shin HS: Macrophage-mediated cytotoxicity: Role of a soluble macrophage cytotoxic factor similar to lymphotoxin and tumor necrosis factor. Proc Natl Acad Sci USA (1983) **80**, 6341-6345.
9. Ortaldo JR, Mason LH, Mathieson BJ, Liang SM, Flick DA and Herberman RB: Mediation of mouse natural cytotoxic activity by tumor necrosis factor. Nature (Lond) (1986) **321**, 700-702.
10. Degliantoni G, Murphy M, Kobayashi M, Francis MK, Perussia B and Trinchieri G: Natural killer (NK) cell-derived hematopoietic colony-inhibiting activity and NK cytotoxic factor. J Exp Med (1985) **162**, 1512-1530.
11. Takeda K, Iwamoto S, Sugimoto H, Takuma T, Kawatani N, Noda M, Masaki A, Morise H, Arimura H and Kondo K: Identity of differentiation inducing factor and tumor necrosis factor. Nature (Lond) (1986) **323**, 338-340.
12. Beutler B, Greenwald D, Hulmes JD, Chang M, Pan YCE, Mathison J, Ulevitch R and Cerami A: Identity of tumor necrosis factor and the macrophage-secreted factor cachectin. Nature (Lond) (1985) **316**, 552-553.
13. Beutler BA, Milsark IW and Cerami A: Cachectin/tumor necrosis factor: Production, distribution and metabolic fate *in vivo*. J Immunol (1985) **135**, 3972-3977.
14. Miyoshi I, Hiraki S, Tsubota T, Kubonishi I, Matsuda Y, Nakayama H and Kimura I: Human B cell, T cell and null cell leukemic cell lines derived from acute lymphatic leukemias. Nature (Lond) (1977) **267**, 843-844.
15. Fukuda S, Ando S, Sanou O, Taniai M, Fujii M, Masaki N, Nakamura K, Ando O, Torigoe K, Sugimoto T and Kurimoto M: Simultaneous production of natural human tumor necrosis factor-α, -β and interferon-α from BALL-1 cells stimulated by HVJ. Lymphokine Res (1988) **7**, 175-185.
16. Naomoto Y, Tanaka N, Fuchimoto S and Orita K: *In vitro* synergistic effect of natural human tumor necrosis factor and natural human interferon-α. Jpn J Cancer Res (1987) **78**, 87-92.
17. Moore GE and Koike E: Growth of human tumor cells *in vitro* and *in vivo*. Cancer (1964) **17**, 11-20.
18. Naomoto Y, Tanaka N, Kondo H, Motoda R, Ando S, Kurimoto M and Orita K: Comparative study between nude mice and immunosuppressed hamsters as recipients of human tumor xenografts. Hiroshima J Med Sci (1987) **36**, 305-308.
19. Nakabayashi H, Taketa K, Miyano K, Yamane T and Sato J: Growth of human cell lines with differenciated functions in chemically defined medium. Cancer Res (1982) **42**, 3858-3863.
20. Ovejera AA, Houchens DP and Baker AD: Chemotherapy of human tumor xenografts in genetically athymic mice. Ann Clin Lab Sci (1978) **8**, 50-56.
21. Fukuda S, Yamane Y, Kouguchi M, Ando S, Sato Y and Kurimoto M: Purification and characterization of natural human tumor necrosis factors from HVJ-stimulated BALL-1 cells. Chem Express (1987) **2**, 101-104.
22. Nobuhara M, Kanamori T, Nagase Y, Nii A, Morishita H, Toyama J, Ando S and Kurimoto M: The expression of human tumor necrosis factor in *E. coli*. Nucleic Acid Res (Symposium series) (1986) **17**, 131-134.
23. Weissmann C: At least three human type interferons: Structure of α-2. Science (1980) **209**, 1343-1347.
24. Ando S, Sanou O, Fujii M, Sato Y and Kurimoto M: Large scale production and characterization of natural human interferon-α from HVJ-stimulated BALL-1 cells. Chem Express (1987) **2**, 45-48.

25. Eifel PJ, Walker SH and Lucas ZJ: Standardization of a sensitive and rapid assay for lymphotoxin. Cell Immunol (1975) **15**, 208-221.
26. Gomi K, Morimoto M and Nakamizo N: Characteristics of antiviral and anticellular activities of human recombinant interferon-γ. Jpn J Cancer Res (1985) **76**, 224-234.
27. Naomoto Y, Kondo H, Tanaka N and Orita K: Novel experimental models of human cancer metastasis in nude mice; Lung metastasis, intraabdominal carcinomatosis with ascites and liver metastasis. J Cancer Res Clin Oncol (1987) **113**, 544-549.
28. Balkwill FR, Lee A, Aldam G, Moodie E, Thomas JA, Tavernier J and Fiers W: Human tumor xenografts treated with recombinant human tumor necrosis factor alone or in combination with interferons. Cancer Res (1986) **46**, 3990-3993.
29. Niitsu Y, Watanabe N, Sone H, Neda H, Yamauchi N and Urushizaki I: Mechanism of the cytotoxic effect of tumor necrosis factor. Jpn J Cancer Res (1985) **76**, 1193-1197.
30. Aggarwal BB, Eessalu TE and Hass PE: Characterization of receptors for human tumor necrosis factor and their regulation by γ-interferon. Nature (Lond) (1985) **318**, 665-667.
31. Ruggiero V, Tavernier J, Fiers W and Baglioni C: Induction of the synthesis of tumor necrosis factor receptors by interferon-γ. J Immunol (1986) **136**, 2445-2450.
32. Tsujimoto M, Yip YK and Vilcěk J: Interferon-γ enhances expression of cellular receptors for tumor necrosis factor. J Immunol (1986) **136**, 2441-2444.
33. Chun M and Hoffmann MK: Combination immunotherapy of cancer in a mouse model: Synergism between tumor necrosis factor and other defence systems. Cancer Res (1987) **47**, 115-118.
34. Haranaka K, Satomi N and Sakurai A: Antitumor activity of murine tumor necrosis factor (TNF) against transplanted murine tumors and heterotransplanted human tumors in nude mice. Int J Cancer (1984) **34**, 263-267.
35. Peters PM, Ortaldo JR, Shalaby MR, Svendersky LP, Nedwin GE, Bringman TS, Hass PE, Aggarwal BB, Herberman RB, Geoddel DV and Palladino MA: Natural killer-sensitive targets stimulate production of TNF-α but not TNF-β (lymphotoxin) by highly purified human peripheral blood large granular lymphocytes. J Immunol (1986) **137**, 2592-2598.
36. Creaven PJ, Plager JE and Dupere S: Phase 1 clinical trial of recombinant human tumor necrosis factor. Cancer Chemother Pharmacol (1987) **20**, 137-144.
37. Fuchimoto S, Itano S, Kimura T, Iwagaki H, Maeda T, Hamada H, Kurimoto M and Orita K: Phase 1 and early phase 2 clinical study of a new antitumor lymphokine produced by stimulated B cell line, BALL-1. Proc Am Soc Clin Oncol (1986) **22**, 910.

Received August 22, 1988; accepted March 7, 1989