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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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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 intraabdominal 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'r'in (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'r'in; HVJ, hemagglutinating virus of Japan; PMA, 4β-phorbol 12β-myristate 13-acetate.

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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 lymphotxin by Eifel et al. (15, 16, 25). The specific activity was 10⁸ U/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 5×10⁸ JRU/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 2.5×10⁴ 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 5×10⁴ 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. Twenty-fifths milliliter of a tumor cell suspension containing 2×10⁴ 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 mm³. Treatment with cytokines was then started. Tumors were observed daily. The tumors were measured weekly with the aid of microlipers, and the tumor volumes were approximated by the formula L×W²/2, 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 2.5×10⁶ 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

<table>
<thead>
<tr>
<th>Cytokines administereda</th>
<th>Number of metastatic nodulesb,c</th>
<th>Range</th>
<th>% Inhibitiond</th>
<th>FBW/IBWe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>194.0 ± 32.7</td>
<td>(85-213)</td>
<td></td>
<td>1.22</td>
</tr>
<tr>
<td>nHuTNF-α</td>
<td>112.3 ± 19.6</td>
<td>(63-162)</td>
<td>42.2</td>
<td>1.25</td>
</tr>
<tr>
<td>nHuIFN-α</td>
<td>87.4 ± 10.6</td>
<td>(44-108)</td>
<td>54.0</td>
<td>1.31</td>
</tr>
<tr>
<td>rHuIFN-γ</td>
<td>95.8 ± 23.6</td>
<td>(50-197)</td>
<td>50.6</td>
<td>1.36</td>
</tr>
<tr>
<td>nHuTNF-α+nHuIFN-α</td>
<td>0 ± 0</td>
<td>(0-0)</td>
<td>100.0</td>
<td>1.20</td>
</tr>
<tr>
<td>nHuTNF-α+rHuIFN-γ</td>
<td>1.3 ± 0.6</td>
<td>(0-3)</td>
<td>99.3</td>
<td>1.23</td>
</tr>
</tbody>
</table>

\(a\): 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, 1×10^4 IU/mouse/day for 21 days; nHuIFN-α, 1×10^4 IU/mouse/day for 21 days; rHuIFN-γ, 5×10^3 IU/mouse/day for 21 days; nHuTNF-α plus nHuIFN-α, administered simultaneously at 1×10^4 U and 1×10^4 IU/mouse/day, respectively, for 21 days; nHuTNF-α plus rHuIFN-γ, 1×10^4 U and 5×10^3 IU/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, \(p<0.001\).

\(d\): % Inhibition = \(\frac{\text{Mean no of metastatic nodules of experiment}}{\text{Mean no of metastatic nodules of control}}\) × 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 $5 \times 10^6$ 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

![Graph](image_url)

**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, $1 \times 10^6$ U/mouse/day for 10 days (○--○); C) nHuIFN-α, $1 \times 10^4$ IU/mouse/day for 10 days (○--○); D) nHuTNF-α plus nHuIFN-α, $1 \times 10^6$ U and $1 \times 10^4$ 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 × (1 - mean $V_t/\bar{V}_o$ ratio of experiment/mean $V_t/\bar{V}_o$ ratio of control), where $V_t$ is the mean tumor volume on day 21 and $\bar{V}_o$ 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 $10^7$ U and $10^7$ IU/mouse/day, respectively, 49.1% at a dose of $10^6$ U and $10^6$ IU/mouse/day, respectively and 7.4% at a dose of $10^5$ U and $10^5$ IU/mouse/day, respectively. Intratumoral combined administration of nHuTNF-α and nHuIFN-α, each at $10^5$ U and $10^5$ IU/mouse/day,

![Graph showing tumor size vs. time after initial treatment](image)

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, $1 \times 10^7$ U and $1 \times 10^7$ IU/mouse/day, respectively, i.v. for 21 days (●). C) nHuTNF-α plus nHuIFN-α, $10^5$ U and $1 \times 10^6$ IU/mouse/day, respectively, i.v. for 21 days (▲). D) nHuTNF-α plus nHuIFN-α, $1 \times 10^4$ U and $1 \times 10^5$ IU/mouse/day, respectively, i.v. for 21 days (■). E) nHuTNF-α plus nHuIFN-α, $1 \times 10^4$ U and $1 \times 10^5$ 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 $V/V_o$, where $V$ is the mean tumor volume on the day of evaluation and $V_o$ 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 10^7 U and 10^7 IU/mouse/day, respectively, exhibited approximately the same degree of growth inhibition as the intratumoral administration at 10^5 U 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 1×10^6 U 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-

![Graphs A to E showing tumor size over time](image)

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, 1×10^4 U and 1×10^4 IU, respectively, daily for 7 days; (▲▲▲▲▲▲), nHuTNF-α plus nHuIFN-α, 1×10^6 U and 1×10^6 IU, 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/V₀, where V is the mean tumor volume on the day of evaluation and V₀ 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 carcinoma).
Fig. 5 Histological appearance of control (saline) (A) and [nHuTNF-α (1×10^4U) plus nHuIFN-α (1×10^4IU)]-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 anticancer activity. It was shown histologically that a single intratumoral injection of the cytokines at doses of 10^4U nHuTNF-α and 10^4IU 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 pycnosis 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 \textit{in vivo} antitumor activity of human cytokines which have species specificity (27). Using these experimental models, we demonstrated in the present study that nHuTNF-\(\alpha\) and nHuIFN-\(\alpha\), as well as nHuTNF-\(\alpha\) and rHuIFN-\(\gamma\), synergistically inhibited metastasis to the lung of RPMI 4788 cells transplanted in nude mice. Treatment with nHuTNF-\(\alpha\) and nHuIFN-\(\alpha\) 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-\(\alpha\) and IFN-\(\beta\) both increase TNF receptors in HeLa cells, albeit with a lower efficacy than IFN-\(\gamma\) (30, 31). Tsujimoto et al. demonstrated that IFN-\(\gamma\) 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-\(\gamma\) together with IFN-\(\beta\) prevented the enhanced binding of TNF seen in cells incubated with IFN-\(\gamma\) alone, whereas the addition of IFN-\(\beta\) did not interfere with the synergistic enhancement of TNF cytotoxicity produced by IFN-\(\gamma\). 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/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-\(\gamma\), 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-\(\alpha\) and nHuIFN-\(\alpha\) 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 \textit{in vitro}, it is puzzling that TNF-\(\alpha\) 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-\(\alpha\) at a high dose because of its cachectin like activity (36). Nevertheless, in the present study, no weight loss was observed in TNF-\(\alpha\) treated mice (Table 1). This absence of weight loss may be due to the low dose of TNF-\(\alpha\) used in the present study. The synergism of nHuTNF-\(\alpha\) and nHuIFN-\(\alpha\) 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-\(\alpha\) and nHuIFN-\(\alpha\) 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).
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