Antitumor effect of natural human tumor necrosis factor-beta against Lewis lung carcinoma in mice and its synergistic potentiation by interferon.

Yoshitaka Nishiyama*  Sadanori Fuchimoto†  Kunzo Orita‡

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Abstract

We investigated the antitumor effect of purified natural human tumor necrosis factor-beta (nHuTNF-beta) produced by human acute lymphoblastic leukemia BALL-1 cells stimulated with HVJ on pulmonary metastatic tumors of Lewis lung carcinoma (3LL) transplanted into BDF1 mice. nHuTNF-beta showed antiproliferative effects on metastatic tumors in a dose-dependent manner when administered daily for 10 days by the intravenous route. Histological examination of the tumors treated with nHuTNF-beta revealed that the tumor size and number of metastases were much reduced. Lytic cellular changes, including cytoplasmic vacuolation, loosening of the intercellular junction and both cytoplasmic and nuclear swelling, were found, but tumor necrosis was not. These findings indicate a therapeutic effect of Grade IIa according to the histological criteria of Shimosato and Ohboshi. In addition, synergistic augmentation of the antiproliferative effects of nHuTNF-beta by natural murine interferon-alpha/beta (nMu-IFN-alpha/beta) or recombinant murine interferon-gamma (rMuIFN-gamma) was recognized by median effect plot analysis. The results suggested that nHuTNF-beta may well deserve clinical trial as a new immunotherapeutical agent for human cancer.

KEYWORDS: tumor necrosis factor, interferon, Lewis lung carcinoma, synergistic potentiation, antitumor effect

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Antitumor Effect of Natural Human Tumor Necrosis Factor-\(\beta\) against Lewis Lung Carcinoma in Mice and Its Synergistic Potentiation by Interferon

Yoshitaka Nishiyama*, Sadanori Fuchimoto and Kunzo Orita
First Department of Surgery, Okayama University Medical School, Okayama 700, Japan

We investigated the antitumor effect of purified natural human tumor necrosis factor-\(\beta\) (nHuTNF-\(\beta\)) produced by human acute lymphoblastic leukemia BALL-1 cells stimulated with HVJ on pulmonary metastatic tumors of Lewis lung carcinoma (3LL) transplanted into BDF1 mice. nHuTNF-\(\beta\) showed antiproliferative effects on metastatic tumors in a dose-dependent manner when administered daily for 10 days by the intravenous route. Histological examination of the tumors treated with nHuTNF-\(\beta\) revealed that the tumor size and number of metastases were much reduced. Lytic cellular changes, including cytoplasmic vacuolation, loosening of the intercellular junction and both cytoplasmic and nuclear swelling, were found, but tumor necrosis was not. These findings indicate a therapeutic effect of Grade IIa according to the histological criteria of Shimosato and Ohboshi. In addition, synergistic augmentation of the antiproliferative effects of nHuTNF-\(\beta\) by natural murine interferon-\(\alpha/\beta\) (nMuIFN-\(\alpha/\beta\)) or recombinant murine interferon-\(\gamma\) (rMuIFN-\(\gamma\)) was recognized by median effect plot analysis. The results suggested that nHuTNF-\(\beta\) may well deserve clinical trial as a new immunotherapeutical agent for human cancer.

Key words: tumor necrosis factor, interferon, Lewis lung carcinoma, synergistic potentiation, antitumor effect

Lymphotoxin (LT) and tumor necrosis factor (TNF) are two types of cytokines produced by activated and appropriately stimulated lymphoid cells. It currently appears that lymphocytes produce LT and TNF while macrophages appear to produce only TNF. LT is a protein originally described by Williams and Granger (1, 2). It is very likely that TNF and LT evolved from a common ancestral gene. Based on the amino acid and cDNA sequences of TNF and LT reported by Pennica et al. and Gray et al. (3, 4), it has been suggested that LT should be termed TNF-\(\beta\) and that the "original" TNF should be designated TNF-\(\alpha\).

There have been relatively few studies on the in vivo antitumor effect of purified TNF-\(\beta\). Recently, it became possible to obtain a large amount of natural human TNF-\(\alpha\) (nHuTNF-\(\alpha\)) as reported by Itano et al. (5).
Furthermore, purified nHuTNF-β can also be produced by the "Hayashibara hamster method" (6) with the use of a human acute lymphoblastic leukemia cell line (BALL-1) treated with hemagglutinating virus of Japan (HVJ) (7, 8). In our present study, the antitumor effect of purified nHuTNF-β was studied with regard to dose-dependent antiproliferation of tumors, histological changes of tumors and the synergistic potentiation of nHuTNF-β by interferon (IFN), with the use of a spontaneous pulmonary metastasis model of Lewis lung carcinoma (3LL).

**Materials and Methods**

**Animals.** Specific pathogen-free female BD (C57BL/6XDBA/2)F1 mice, aged 8 to 9 weeks, were purchased from Shizuoka Laboratory Animal Center, Shizuoka, Japan. They were housed under a conventional environment and fed with normal mouse pellet and tap water ad libitum.

**Tumor.** Lewis lung carcinoma (3LL) cells were serially passed subcutaneously in C57BL/6 mice at the First Department of Surgery, Okayama University Medical School. For the experiment, tumors were excited aseptically on the 10th day after the last transplantation, minced, washed three times with Hanks solution, treated with 0.25% trypsin (Difco Labs, Detroit, MI, USA) at 37°C for 15 min, washed twice with Eagle's minimal essential medium (MEM, GIBCO, Labs, Grand Island, NY, USA) supplemented with 10% fetal calf serum (GIBCO Labs), filtered twice through #150 wire mesh and prepared into single cells (4×10^2 cells/ml). Viability of the cells was over 95%.

**Reagents.** Natural human TNF-β (nHuTNF-β) derived from human acute lymphoblastic leukemia BALL-1 cells was supplied by Hayashibara Biochemical Laboratories, Inc., Okayama, Japan (7, 8). nHuTNF-β is a protein consisting of 171 amino acids with a molecular weight of 17,000 and isoelectric point of 6.5-7.0. It is stable at 56°C for 30 min. Its specific activity was 4×10^8 U/mg of protein. The cytotoxicity of this preparation was determined by the highly sensitive and rapid assay of Eifel et al. for lymphotoxin, using mouse L929 cells as the target cells (9). One unit of activity is defined as the reciprocal of the highest dilution that induces a cytopathic effect (CPE) in 50% of the target cells.

Natural murine IFN-α/β (nMuIFN-α/β) was derived from mouse L202 cells, a cloned cell line of L929 cells. This interferon was produced at Hayashibara Biochemical Laboratories, Inc., by the methods previously reported by Kawade et al. (10). Its specific activity was 1×10^7 IU/mg of protein.

Recombinant murine IFN-γ (rMulFN-γ) was generously provided by Shionogi Pharmaceutical Co., Ltd., Osaka, Japan (11). Its specific activity was 3-5×10^4 IU/mg of protein, and this preparation contained less than 2 ng of endotoxin/mg of protein as assayed by the Limulus test.

**Spontaneous pulmonary metastasis model.** As reported by Itano et al. (5), a 0.025 ml aliquot of 3LL cells (1×10^6 cells) was inoculated to the left footpad of BDF1 mice, and the tumors which formed were regarded as the primary tumors (12). They were removed by femoral amputation under ether anesthesia on the 10th day from the inoculation. On the 21st day from the inoculation, the mice were killed with ether and weighed. After that, the metastatic pulmonary tumors were counted by Wexler’s method (13). In brief, their lungs were excised in one piece, dyed with India ink, washed for 5 min with tap water, bleached and fixed in Fekete’s solution for 24 h, and then the metastases were counted with the naked eye. The mean values and standard deviation (SD) of each group were calculated. The following equation was used to determine the inhibition rate of metastasis:

\[
\text{Inhibition rate} (\%) = \left( \frac{\text{mean No. of pulmonary metastases in experimental group}}{\text{mean No. of pulmonary metastases in control group}} - 1 \right) \times 100.
\]

**Dose-dependent antiproliferative effect of nHuTNF-β.** From the day after femoral amputation, namely on the 11th day from inoculation into the left footpad, saline or nHuTNF-β was administered daily for 10 days into the tail veins of mice. The dose of nHuTNF-β ranged between 10^5 and 10^6 U/kg/day.

**Synergistic antiproliferative effects of nHuTNF-β with nMuIFN-α/β or rMulFN-γ.** From
Antitumor Effect of Human Tumor Necrosis Factor-β

the day after femoral amputation, saline or nHu-TNF-β with or without nMulfFN-a/β or rMulfFN-γ was injected daily for 10 days into the tail veins of mice. The doses of these cytokines ranged between $10^2$ and $10^4$ U/kg/day of nHuTNF-β, $10^2$ and $10^4$ IU/kg/day of nMulfFN-a/β, or $10^1$ and $10^3$ IU/kg/day of rMulfFN-γ.

**Histological examinations.** We histologically examined the antitumor effect of nHuTNF-β on metastatic pulmonary tumors. After the lungs were fixed with 10% formalin solution, five samples out of each group were chosen at random. One section cut from each sample at a distance of 5 mm from the trachea was dyed with hematoxylin and eosin solution. The metastatic foci of each section were enumerated at 5 times magnification. The longest and shortest diameters were measured and plotted. We evaluated the therapeutic effect according to the criteria of Shimosato and Ohboshi at 100 times magnification (14).

**Statistical analysis and estimation of synergism.** Statistical analysis was carried out by Student’s $t$-test. Because the median effect plot analysis (15) is relatively simple and is valid whether the effects of the two cytokines are mutually exclusive or nonexclusive, we used it for the assessment of synergism. First, from the dose-effect response, we produced a median effect plot according to:

$$\text{Fa}/(1 - \text{Fa}) = (D/Dm)^m,$$

where Fa is the fraction of the system affected (This means the inhibition rate in our experiments.), D is the dose used, Dm is the dose required to produce the median effect, and m is a Hill-type coefficient. Second, with respect to the value of the Fa, we calculated the combination index (CI) according to:

$$\text{CI} = D_3/D_1 + D_3/D_2 + (D_3 \times D_3)/(D_1 \times D_2),$$

where D1 is the dose of one cytokine, D2 is the dose of the other cytokine, and D3 is the combined dose. We drew a graphical presentation of the CI by computer. If the CI was < 1.0, the interaction of the two reagents was judged to be synergistic. Otherwise, it was judged to be additive, antagonist, or null.

**Results**

**Antiproliferative effect of nHuTNF-β on 3LL in BDF1 mice and the variation in the body weights of mice.** Table 1 shows the growth inhibition effect of nHuTNF-β at a dose of $10^2$ to $10^7$ U/kg/day when intravenously administered for 10 consecutive days. Inhibition rates of 24.3% and 65.4% were obtained at doses of $10^2$ and $10^7$ U/kg/day, respectively. Between a dose of $10^2$ and $10^7$ U/kg/day, nHuTNF-β had an antiproliferative effect in a dose-dependent manner. Significant antiproliferative effect was shown when the dose was more than $10^3$ U/kg/day ($p < 0.01$). No variation was found in the body weights of mice between the control group and the nHuTNF-β treated group.

<table>
<thead>
<tr>
<th>Dose of nHuTNF-β (U/kg/day)$a$</th>
<th>No. of metastases$^b$</th>
<th>Inhibition rate (%)$^c$</th>
<th>P values</th>
<th>Body weight (g)$^d$</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>25.5 ± 5.3</td>
<td>—</td>
<td>—</td>
<td>19.4 ± 0.7</td>
<td>—</td>
</tr>
<tr>
<td>$10^2$</td>
<td>19.3 ± 6.9</td>
<td>24.3</td>
<td>N.S.$^d$</td>
<td>19.0 ± 0.7</td>
<td>N.S.$^d$</td>
</tr>
<tr>
<td>$10^3$</td>
<td>14.3 ± 3.6</td>
<td>43.8</td>
<td>&lt; 0.01</td>
<td>19.2 ± 0.5</td>
<td>N.S.</td>
</tr>
<tr>
<td>$10^4$</td>
<td>14.0 ± 2.8</td>
<td>45.1</td>
<td>&lt; 0.01</td>
<td>19.7 ± 0.4</td>
<td>N.S.</td>
</tr>
<tr>
<td>$10^5$</td>
<td>12.7 ± 7.5</td>
<td>50.3</td>
<td>&lt; 0.01</td>
<td>19.3 ± 0.4</td>
<td>N.S.</td>
</tr>
<tr>
<td>$10^6$</td>
<td>10.3 ± 3.9</td>
<td>59.5</td>
<td>&lt; 0.01</td>
<td>19.5 ± 0.3</td>
<td>N.S.</td>
</tr>
<tr>
<td>$10^7$</td>
<td>8.8 ± 7.7</td>
<td>65.4</td>
<td>&lt; 0.01</td>
<td>19.2 ± 0.7</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

$a$: nHuTNF-β was administered into the tail veins of 6 mice in each group at the indicated dose daily for 10 days.

$b$: Mean ± SD.

c: Calculated by the equation described in Materials and Methods section.

d: N.S.: not significant by Student’s $t$-test.

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Effects of nHuTNF-β on the size and number of metastatic pulmonary tumors. The sizes of individual metastatic pulmonary tumors in each group (saline and nHuTNF-β at a dose of $10^7$ U/kg/day) were plotted in Fig. 1. In the control group, 30 metastatic nodules were found, with sizes ranging between $1.0 \times 0.9$ mm and $3.9 \times 2.8$ mm. Conversely, in the nHuTNF-β ($10^7$ U/kg/day) administered group, there were 7 metastatic nodules whose sizes ranged from $0.8 \times 0.6$ mm to $2.1 \times 1.6$ mm. In brief, the distribution of tumors in the control group showed a biphasic pattern (larger and smaller tumor size), while that in the nHuTNF-β administered group showed a uniphasic pattern (smaller tumor size), which was suggestive of antiproliferation due to nHuTNF-β.

Histological examination of pulmonary metastatic tumors in the control group and nHuTNF-β administered group. Fig. 2A demonstrates the histology of pulmonary metastatic tumors on 21 days after tumor inoculation in the control group. The intercellular junction was tight and each tumor cell proliferated remarkably. Some mitotic cells were found in the specimen. Fig. 2B shows the histology of pulmonary metastatic tumors after 10 consecutive days of intravenous administration of nHuTNF-β at a dose of $10^7$ U/kg/day. In the nHuTNF-β administered group, cytoplasmic and nuclear swelling, and

![Graph](http://escholarship.lib.okayama-u.ac.jp/amo/vol43/iss1/3)
Fig. 2  Histological examinations of pulmonary metastatic tumors in the control group (2A) and nHuTNF-β administered group (2B) (100×, H.E. staining). From the day after removal of primary tumors (3LL cells transplanted into the left footpad), nHuTNF-β (1×10^7 U/kg/day) was injected intravenously daily for 10 days. On day 21, mice were killed, and specimens of lung tumors were prepared. Bar = 20 μm.
Table 2  Synergistic antiproliferative effects of natural human tumor necrosis factor-β (nHuTNF-β) in combination with natural murine interferon-α/β (nMuIFN-α/β) on Lewis lung carcinoma in BDF1 mice

<table>
<thead>
<tr>
<th>Dose of cytokinesa</th>
<th>No. of metastasesb</th>
<th>Inhibition rate (%)c</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2e</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3f</td>
</tr>
<tr>
<td>Saline</td>
<td>30.6 ± 5.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>nMuIFN-α/β, 10²</td>
<td>23.2 ± 4.1</td>
<td>24.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>nHuTNF-β, 10³</td>
<td>22.4 ± 7.8</td>
<td>26.8</td>
<td>N.S.</td>
</tr>
<tr>
<td>nMuIFN-α/β, 10³</td>
<td>17.4 ± 3.8</td>
<td>43.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>+ nHuTNF-β, 10³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16.8 ± 4.1</td>
<td>45.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>nMuIFN-α/β, 10³</td>
<td>15.0 ± 4.0</td>
<td>51.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>nMuIFN-α/β, 10³</td>
<td>8.8 ± 2.2</td>
<td>71.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>+ nHuTNF-β, 10³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nMuIFN-α/β, 10⁴</td>
<td>11.0 ± 2.5</td>
<td>64.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>nHuTNF-β, 10⁴</td>
<td>13.0 ± 2.5</td>
<td>57.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>nMuIFN-α/β, 10⁴</td>
<td>6.0 ± 4.8</td>
<td>80.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>+ nHuTNF-β, 10⁴</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a: Drugs were administered into the tail veins of 5 mice in each group singly or in combination at the indicated dose (nHuTNF-β: U/kg/day, nMuIFN-α/β: IU/kg/day) daily for 10 days.
b: Mean ± SD.
c: Calculated by the equation described in Materials and Methods section.
d: Compared with saline control group.
e: Compared with nMuIFN-α/β administered group.
f: Compared with nHuTNF-β administered group.
†: Not significant.

cytoplasmic vacuolization were found. Also, there were some dead cells without nuclei. The intercellular junction became loose. This less distinct cellular junction seemed to be caused by nHuTNF-β. Nevertheless, there were no hemorrhagic tumor necrosis and little lymphocyte infiltration. The histological grading of the therapeutic effect according to the criteria of Shimosato and Oboshi was Grade IIa.

Synergistic antiproliferative effects of nHuTNF-β in combination with nMuIFN-α/β on 3LL in BDF1 mice. nHuTNF-β, nMuIFN-α/β or both of them were administered at doses between 10² and 10⁴ U/kg/day (or IU/kg/day) at a dose ratio of 1:1 into the tail veins of mice daily for 10 days beginning 11 days after tumor inoculation. The combined antiproliferative effects against tumors are shown in Table 2. A dose of

10² U/kg/day nHuTNF-β and 10² IU/kg/day nMuIFN-α/β gave rise to a 24.2% and 26.8% inhibition rate, respectively, but in combination, the inhibition rate was potentiated up to 43.1%. Similar augmentation was recognized with doses of 10³ and 10⁴ U/kg/day of nHuTNF-β. Statistical analysis revealed significant differences (p < 0.05 or p < 0.01).

Synergistic antiproliferative effects of nHuTNF-β in combination with rMuIFN-γ on 3LL in BDF1 mice. nHuTNF-β, rMuIFN-γ or both of them were injected at a dose ratio of 1:1/10 into the tail veins of mice daily for 10 days beginning the day after femoral amputation. The combined antiproliferative effects against tumors are shown in Table 3. A dose of 10² U/kg/day nHuTNF-β and 10 IU/kg/day rMuIFN-γ gave rise to the inhibition rates of 28.1% and 31.8%, respec-
Table 3  Synergistic antiproliferative effects of natural human tumor necrosis factor-β (nHuTNF-β) in combination with recombinant murine interferon-γ (rMuIFN-γ) on Lewis lung carcinoma in BDF1 mice

<table>
<thead>
<tr>
<th>Dose of cytokines</th>
<th>No. of metastases</th>
<th>Inhibition rate (%)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td></td>
<td>1*</td>
</tr>
<tr>
<td>rMuIFN-γ, 10^4</td>
<td>36.2 ± 4.9</td>
<td>26.0 ± 4.1</td>
<td>28.2</td>
</tr>
<tr>
<td>nHuTNF-β, 10^3</td>
<td>24.7 ± 3.3</td>
<td>31.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>rMuIFN-γ, 10^4</td>
<td>16.3 ± 7.0</td>
<td>55.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>+ nHuTNF-β, 10^3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rMuIFN-γ, 10^3</td>
<td>20.5 ± 7.4</td>
<td>43.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>nHuTNF-β, 10^4</td>
<td>20.8 ± 5.4</td>
<td>42.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>+ rMuIFN-γ, 10^4</td>
<td>11.8 ± 1.7</td>
<td>67.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>rMuIFN-γ, 10^4</td>
<td>18.0 ± 7.6</td>
<td>50.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>nHuTNF-β, 10^4</td>
<td>18.2 ± 6.0</td>
<td>49.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>+ rMuIFN-γ, 10^4</td>
<td>7.8 ± 2.6</td>
<td>78.5</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

a: Drugs were administrated into the tail veins of 6 mice in each group singly or in combination at the indicated dose (nHuTNF-β: U/kg/day, rMuIFN-γ: IU/kg/day) daily for 10 days.
b: Mean ± SD.
c: Calculated by the equation described in Materials and Methods section.
d: Compared with saline control group.
e: Compared with rMuIFN-γ administrated group.
f: Compared with nHuTNF-β administrated group.
g: N.S.: not significant.

Effectively, but in combination, the inhibition rate was increased to 55.0%. Similar augmentation was recognized with doses of 10^3 and 10^4 U/kg/day of nHuTNF-β. Statistical analysis revealed a significant difference (p < 0.01).

Median effect plot and graphical presentation of the CI with respect to the fraction affected (Fa) of the synergistic inhibition by nHuTNF-β and nMuIFN-α/β or rMuIFN-γ. The median effect plots for nHuTNF-β together with nMuIFN-α/β or rMuIFN-γ (Figs. 3A and 3C) were obtained from the data in Tables 2 and 3, respectively. The graphical presentations of the CIs in Figs. 3A and 3C are shown in Figs. 3B and 3D, respectively. In relation to nHuTNF-β, nMuIFN-α/β and the combination, the median effect equations were

\[ \log \left[ \frac{1}{1 - Fa} \right] = 0.37 \log D - 1.23 \]

and

\[ \log \left[ \frac{1}{1 - Fa} \right] = 0.37 \log D - 0.81 \]

respectively. The CI was calculated to be 0.158 (<1.0), and the combined effect was judged to be synergistic. Likewise, the equations of the median effect plots in relation to nHuTNF-β, rMuIFN-γ and the combination were

\[ \log \left[ \frac{1}{1 - Fa} \right] = 0.16 \log D - 0.64, \]

\[ \log \left[ \frac{1}{1 - Fa} \right] = 0.21 \log D - 0.58 \]

and

\[ \log \left[ \frac{1}{1 - Fa} \right] = 0.24 \log D - 0.39, \]

respectively. The CI was calculated to be 0.035 (<1.0). The combined effect was judged to be synergistic.
Fig. 3 Median effect plot and graphical presentation of the combination index (CI) with respect to the fraction affected (Fa) for the synergistic inhibition by nHuTNF-β and rMolFN-α/β or rMolFN-γ. A: median effect plot for nHuTNF-β (●), rMolFN-α/β (○) and the combination (×). C: median effect plot for nHuTNF-β (●), rMolFN-γ (○) and the combination (×). B and D: graphical presentation of the CI in Figs. 3A and 3C, respectively.
Discussion

Williams and Granger first reported that a soluble factor from PHA-stimulated lymphocytes was cytotoxic to mouse L cells (1, 2). Hence, it was termed "lymphotoxin" (LT), and the antitumor effect of crude LT was investigated in vitro and in vivo. Gray et al. described the purification and the amino acid sequences of RPMI 1788 cell-derived LT (3). LT has a 28% amino acid sequence homology with TNF; the genes of LT and TNF are linked on human chromosome No. 6 (chromosome No. 17 in the mouse)(16); LT and TNF share a common receptor on ME-180 human cervical cancer cells (17), and they have the same biological properties. TNF-α has recently become available for clinical use as a recombinant type and the broad spectrum of its biological activities has been studied in vitro and in vivo. However, there are few reports on the antitumor effect of purified TNF-β (synonymous with LT). Therefore, we studied the antitumor effect of nHuTNF-β derived from BALL-1 cells stimulated with HVJ with the use of a 3LL pulmonary metastasis model.

nHuTNF-β was shown to have a dose-dependent antiproliferative effect in the dose range of 10^2 to 10^7 U/kg/day when intravenously administered for 10 consecutive days. Both the number and size of metastatic tumors were reduced. On the other hand, cellular and nuclear swelling, loosening of the cell-cell connection and intracellular vacuolation were recognized, but tumor necrosis and infiltration of lymphocytes reported by others were not found (18). Because we administered nHuTNF-β into the tail vein and not into the tumor, and used a lower dose of nHuTNF-β than that reported previously (18), the cells may have been gradually affected without rapid tumor necrosis.

Recently, in vivo mechanisms of the cytotoxicity of TNF-α have been reported by many investigators. For instance, it has been suggested that TNF-α acts on endothelial cells of vessels, induces production of interleukin-1 (IL-1), and enhances both procoagulant production (19) and neutrophilic adherence (20). With regard to indirect antitumor effects, Ransom et al. (21) showed that LT specifically increased the sensitivity of tumor cells to natural killer (NK) cells but not to macrophage and that the lymphokine suppressed tumor formation in vivo. Though we studied the NK cellular activity in a 4-h ^51Cr release assay with splenic cells of mice which were administered nHuTNF-β for 10 days at a dose of 1×10^7 U/kg/day, no augmentation of NK cytotoxicity was recognized (data not shown). Because Ransom et al. used crude LT, IFN-γ in these preparations might have enhanced cytotoxicity by NK cells.

In the past, some researches on the synergism between IFN and LT have been reported (22, 23). However, purified LT was not used in these studies. Judging from Tables 2 and 3 and Fig. 3, synergistic potentiation of the antitumor effect of nHuTNF-β by interferon was apparent. However, the mechanism whereby interferon enhances the effect of TNF-β remains to be determined. Concerning TNF-α, Itano et al. reported that synergism between natural human TNF-α and murine interferon-α/β or interferon-γ was recognized in vivo (24). Also, Naomoto et al. reported synergism between natural human TNF-α and natural human interferon-α in vitro (25). Similar mechanisms might be considered because TNF-α and -β share a common receptor and IFN-γ enhances the number of TNF receptors without changing its affinity.

In the future, comparison of the antitumor effect between TNF-α and TNF-β should be investigated in addition to the analysis of the mechanism of the antitumor effect induced by TNF-β. Based on the present study using an
animal model, a clinical trial of nHuTNF-β in combination with IFN is worth consideration.

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