Enhancement of experimental metastasis by gamma-interferon in a murine adenocarcinoma.

Tetsuo Hirano*  Akio Hizuta†
Noriaki Tanaka‡  Kunzo Orita**

*Okayama University,
†Okayama University,
‡Okayama University,
**Okayama University,
Enhancement of experimental metastasis by gamma-interferon in a murine adenocarcinoma.*

Tetsuo Hirano, Akio Hizuta, Noriaki Tanaka, and Kunzo Orita

Abstract

This study was conducted to examine the effect of gamma-interferon (IFN-gamma) on experimental metastasis formation by murine colon 26 adenocarcinoma in BALB/c mice. We found that the number of experimental lung metastases was increased after colon 26 cells were pretreated for 1 h with as little as 1 OIU/ml of IFN-gamma. 5-[125I] iodo-2'-deoxyuridine-radiolabeled colon 26 cells pretreated with IFN-gamma remained at higher level in the lung at 24h after intravenous injection than when the cells were not pretreated. In vivo elimination of asialo GM1-positive cells increased the number of lung metastases and, in such mice, there was no longer a difference in metastatic ability between control and IFN-gamma-treated cells. Colon 26 cells were completely resistant to lysis by isolated splenocytes. Splenocytes incubated in vitro with interleukin 2 exhibited moderate cytotoxicity against colon 26 cells, but there were no significant differences between control and IFN-gamma-treated cells. Colon 26 cells pretreated with IFN-gamma demonstrated resistance to tumor necrosis factor alpha-mediated growth inhibition. The enhancement of metastases by IFN-gamma was dependent on de novo protein synthesis since the enhancement was abolished by cycloheximide. Taken together, the data suggest that the metastatic ability of colon 26 cells pretreated with IFN-gamma is significantly higher due to the resistance to asialo GM1-positive cells accompanied with de novo protein synthesis.

KEYWORDS: IFN-gamma, colon 26 murine adenocarcinoma, lung metastasis

*PMID: 8701776 [PubMed - indexed for MEDLINE]
Copyright (C) OKAYAMA UNIVERSITY MEDICAL SCHOOL
Enhancement of Experimental Metastasis by γ-Interferon in a Murine Adenocarcinoma

Tetsuo Hirano, Akio Hizuta*, Noriaki Tanaka and Kunzo Orita
First Department of Surgery, Okayama University Medical School, Okayama 700, Japan

This study was conducted to examine the effect of γ-interferon (IFN-γ) on experimental metastasis formation by murine colon 26 adenocarcinoma in BALB/c mice. We found that the number of experimental lung metastases was increased after colon 26 cells were pretreated for 1h with as little as 10IU/ml of IFN-γ. 5-125I iodo-2'-deoxyuridine-radiolabeled colon 26 cells pretreated with IFN-γ remained at higher level in the lung at 24h after intravenous injection than when the cells were not pretreated. In vivo elimination of asialo GM1-positive cells increased the number of lung metastases and, in such mice, there was no longer a difference in metastatic ability between control and IFN-γ-treated cells. Colon 26 cells were completely resistant to lysis by isolated splenocytes. Splenocytes incubated in vitro with interleukin 2 exhibited moderate cytotoxicity against colon 26 cells, but there were no significant differences between control and IFN-γ-treated cells. Colon 26 cells pretreated with IFN-γ demonstrated resistance to tumor necrosis factor α-mediated growth inhibition. The enhancement of metastases by IFN-γ was dependent on de novo protein synthesis since the enhancement was abolished by cycloheximide. Taken together, the data suggest that the metastatic ability of colon 26 cells pretreated with IFN-γ is significantly higher due to the resistance to asialo GM1-positive cells accompanied with de novo protein synthesis.

Key words: γ-interferon, colon 26 murine adenocarcinoma, lung metastasis

Interferons (IFNs) comprise a family of proteins divided into three types, identified as α, β, and γ. They each have distinct peptide sequences and a range of biological functions, including antiviral, antiproliferative, immunomodulatory, and differentiation effects (1, 2). A number of authors have reported that the immunomodulatory effect of IFN-γ is different from that of IFN-α or IFN-β and also that IFN-γ influence a number of biological processes occurring during tumor development (3, 4). Recently, several studies have confirmed that pretreatment of tumor cells with IFN-γ increases number of metastatic loci (5-9). In this study, we demonstrate that pretreatment with IFN-γ significantly enhances the metastatic potential of colon 26 cells and investigate the mechanism of this enhancement.

Materials and Methods

Mice. Specific pathogen free male BALB/c mice were housed under conventional conditions and were used at 6-8 weeks of age.

Tumor cells. Colon 26 adenocarcinoma cells were derived from colorectal adenocarcinoma as previously reported (10). The cells were maintained in monolayer cultures in RPMI-1640 containing 10% fetal calf serum and prepared in phosphate buffered saline (PBS) by trypsinization before use.

Cytokines and pretreatment of tumor cells. Recombinant murine IFN-γ and recombinant human tumor necrosis factor α (TNF-α) were kindly provided by Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). Their specific activities were 1.5 × 10^7 U/mg and 1 × 10^6 U/mg protein, respectively. Colon 26 tumor cells were pretreated with 100IU/ml IFN-γ for 1h at 37 °C unless otherwise indicated. The cells were washed 3 times, resuspended in PBS and used for the metastasis assay. In the experiment with cycloheximide treatment, colon 26 cells were incubated with 50

* To whom correspondence should be addressed.
μg/ml of cycloheximide (Sigma Chemical Co., St. Louis, MO, USA) for 1 h before IFN-γ treatment.

**Experimental metastasis assay.** A 0.2-ml aliquot of single-cell suspension containing 5 × 10⁴ colon 26 cells pretreated with IFN-γ was injected into mice via a lateral tail vein with a 27-gauge needle. Twenty-one days later, the mice were sacrificed under deep ether anesthesia and lungs were removed to count the number of surface nodules.

**Retention of radiolabeled tumor cells in the lungs.** Cultures of colon 26 cells in mid-log phase were prelabeled with 5-[^125]I iodo-2′-deoxyuridine (Amersham International, Buckinghamshire, United Kingdom) (specific activity, 5 Ci/mg) at 0.3 μ Ci/ml of medium for 24 h (11). During the last 1 h, colon 26 cells were treated with 100 IU/ml of IFN-γ. Following trypsinization, the cell suspension was washed 3 times and resuspended at a final concentration of 2.5 × 10⁶ cells/0.2 ml of PBS. Labeled tumor cells were injected via a tail vein into male BALB/c mice. The mice were sacrificed by deep ether anesthesia at the times indicated after tumor cell injection (1 h, 4 h, or 24 h). Lungs were immediately collected from each mouse and washed in 3 changes of 70% ethanol over a 72-h period to remove free label (11), and radioactivity was counted in a gamma counter.

**Treatment with anti-asialo GM₁ antibody.** Mice received a single injection via a tail vein of either rabbit anti-asialo GM₁ polyclonal antibody (anti-AGM, Ab; 100 μg in 200 μl PBS; Wako Pure Chemical Industries, Ltd., Osaka, Japan) or PBS 18 h before tumor cell injection.

**Splenocytes.** Spleens were removed aseptically and gently crushed in RPMI-1640 containing 10% fetal calf serum. The cells were passed through nylon mesh and then placed in buffered ammonium chloride solution to produce osmotic lysis of erythrocytes. The splenocytes were then centrifuged and washed 3 times.

**Preparations of lymphokine-activated killer (LAK) cells.** Fresh splenocytes were incubated in recombinant human interleukin 2 (IL-2) (1 × 10⁵ IU/ml) in complete medium at a concentration of 2.5 × 10⁸ cells/ml for 96 h at 37 °C in a moist atmosphere with 5% CO₂.

**Cytotoxicity assay.** Colon 26 cells were labeled with Na₂[^51]CrO₄ ([⁵¹]Cr; New England Nuclear, Boston, MA, USA) for 1 h. After washing 3 times, the colon 26 target cells were added at a concentration of 1 × 10⁴ cells/well to various numbers of effector cells in triplicate in 96-well round-bottomed microtiter plates in final volume of 200 μl. After 4 h of incubation, the radioactivity in 100 μl of supernatant was determined in a gamma counter and percent specific lysis was calculated as

\[
\text{Experimental cpm} - \text{spontaneous cpm} \times 100
\]

\[
\text{Maximum cpm} - \text{spontaneous cpm}
\]

**Osmotic fragility.** This was determined using the method of Weiss et al. (12). Cells (5 × 10⁵) were incubated for 1 h at 37 °C with ^⁵¹Cr and washed 3 times with Hank's balanced salt solution (HBSS). Cells (10⁵) in 10-μl aliquots were added to the U-bottomed wells of microtiter plates containing 200-μl aliquots of various concentrations of HBSS ranging from 100 % to 0 % for 30 min. Supernatant radioactivity was determined and percent specific lysis was calculated using the same formula as for the cytotoxicity assay.

**Growth inhibition assay.** Colon 26 cell growth was determined using the previously described 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (13). Colon 26 cells (5,000 cells/well in a volume of 100 μl) were cultured in 96-well flat-bottomed microtiter plates. After 24 h, colon 26 cells were treated with control diluent or IFN-γ (100 IU/ml) for 1 h and washed 3 times. Various concentrations of TNF-α were added and cultured for 3 days. At the end of the incubation, 20 μl of MTT (Sigma Chemical Co.) (5 mg/ml) were added to all wells. After 4 h of incubation at 37 °C, the MTT was removed from all wells and the formazan crystals in the adherent colon 26 cells were solubilized with 100 μl 0.04 N isopropanol HCl. Plates were agitated gently and spectrophotometric absorbance at 570 nm was read using Easy Reader EAR 340 (SLT-Labinstruments, Austria). Percent growth inhibition was calculated as

\[
100 - \frac{\text{Experimental absorbance}}{\text{Control absorbance}} \times 100
\]

**Statistics.** Statistical analyses of these data were performed using Student's t-test.

**Results.**

**Enhancement of metastasis by IFN-γ pretreatment.** Table 1 demonstrates that pretreatment of colon 26 cells for 1 h with IFN-γ significantly increased the number of metastatic loci in the lungs of...
Hirano et al.: Enhancement of experimental metastasis by IFN-γ

Table 1  Enhancement of metastasis by interferon-γ (IFN-γ) pretreatment

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Pretreatment of tumor cells</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medium</td>
<td>IFN-γ&lt;sup&gt;αβ&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>21.4 ± 8.9&lt;sup&gt;α&lt;/sup&gt;</td>
<td>76.2 ± 9.4&lt;sup&gt;α&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>26.4 ± 7.4</td>
<td>63.8 ± 8.8&lt;sup&gt;α&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>5.2 ± 5.2</td>
<td>66.4 ± 9.4&lt;sup&gt;α&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>α</sup> Colon 26 cells were pretreated with 100IU/ml IFN-γ for 1h.
<sup>β</sup> Data represent number of lung metastases. Values are the mean ± SD of five mice.
* P < 0.0001 versus medium control.

Table 2  Dose dependence of IFN-γ on metastasis

<table>
<thead>
<tr>
<th>IFN-γ pretreatment (IU/ml)&lt;sup&gt;αβ&lt;/sup&gt;</th>
<th>No. of lung metastases&lt;sup&gt;α&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>48 ± 5.2</td>
</tr>
<tr>
<td>1</td>
<td>10.2 ± 3.4</td>
</tr>
<tr>
<td>10</td>
<td>14.2 ± 8.3</td>
</tr>
<tr>
<td>10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>39.0 ± 35.4</td>
</tr>
<tr>
<td>10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>38.8 ± 4.4</td>
</tr>
</tbody>
</table>

<sup>α</sup> Colon 26 cells were pretreated for 1 h with IFN-γ at the concentrations indicated. INF-γ: spelled out in Table 1.
<sup>β</sup> Values are the mean ± SD of five mice.
* P < 0.05;  ** P < 0.001;  *** P < 0.0001.

BALB/c mice.

Dose dependence of IFN-γ on metastasis. As shown in Table 2, a 1-h incubation of colon 26 cells with as little as 10IU/ml significantly increased the number of surface lung nodules. Incubation of colon 26 cells with increasing concentrations of IFN-γ resulted in a significantly greater enhancement.

Pulmonary retention of colon 26 cells. To investigate the mechanisms responsible for the IFN-γ-mediated increase of experimental metastases, we examined the pulmonary retention of radiolabeled colon 26 cells pretreated with control diluent or IFN-γ at 1h, 4h, or 24h after tumor cell injection (Table 3). After intravenous injection, radiolabeled tumor cells rapidly localized in the lung, and no difference was observed between the two groups in the first 4h. At 24h, however, the radioactivity in the lungs of mice injected with colon 26 cells pretreated with IFN-γ was significantly greater than in those injected with colon 26 cells incubated with control diluent.

Effect of anti-AGM<sub>1</sub> Ab treatment on metastasis. In BALB/c mice, the natural killer (NK) activity of isolated splenocytes was completely abolished for 72h after intravenous injection of 100μg anti-AGM<sub>1</sub> Ab (100μg in 200μl PBS) (data not shown). There were significantly more metastatic loci in either group of colon 26 cells pretreated with control diluent or IFN-γ in anti-AGM<sub>1</sub> Ab treated mice compared with their immunocompetent counterparts. However, in anti-AGM<sub>1</sub> Ab-treated mice, colon 26 cells pretreated with IFN-γ showed the same metastatic ability as untreated cells (Table 4).

Effect of IFN-γ on susceptibility to LAK cell-mediated lysis. In a standard 4h cytotoxicity assay, colon 26 cells were completely resistant to lysis by freshly isolated splenocytes (data not shown). Splenocytes incubated in vitro in IL-2 (LAK cells) demonstrated moderate cytotoxicity against colon 26 cells, but there were no significant differences between the control and IFN-γ groups (Table 5).

Effect of IFN-γ on osmotic fragility.

Table 3  Pulmonary retention of colon 26 cells

<table>
<thead>
<tr>
<th>Pretreatment of tumor cells</th>
<th>Time after tumor cell injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1h</td>
</tr>
<tr>
<td>PBS</td>
<td>62.0&lt;sup&gt;αβ&lt;/sup&gt;</td>
</tr>
<tr>
<td>IFN-γ&lt;sup&gt;αβ&lt;/sup&gt;</td>
<td>66.9</td>
</tr>
</tbody>
</table>

<sup>α</sup> Data represent % of radioactivity remaining in the lung. Values are the mean ± SD of five mice.
<sup>β</sup> Colon 26 cells were pretreated with 100IU/ml IFN-γ for 1h.
* P < 0.001 versus PBS control.
PBS: phosphate buffered saline; IFN-γ: spelled out in Table 1.

Table 4  Effect of anti-asialo GM<sub>1</sub> treatment on lung metastasis

<table>
<thead>
<tr>
<th>Treatment of mice</th>
<th>Pretreatment of tumor cells</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medium</td>
<td>IFN-γ&lt;sup&gt;αβ&lt;/sup&gt;</td>
</tr>
<tr>
<td>PBS</td>
<td>64 ± 4.4&lt;sup&gt;α&lt;/sup&gt;</td>
<td>22.8 ± 9.4&lt;sup&gt;α&lt;/sup&gt;</td>
</tr>
<tr>
<td>Anti-asialo GM&lt;sub&gt;1&lt;/sub&gt;</td>
<td>44.6 ± 9.0</td>
<td>44.4 ± 11.0&lt;sup&gt;α&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>α</sup> Colon 26 cells were pretreated with 100IU/ml IFN-γ for 1h.
<sup>β</sup> Data represent number of lung metastases. Values are the mean ± SD of five mice.
* P < 0.01 versus medium control.
PBS, IFN-γ: spelled out in Tables 1 and 3.
Both control and IFN-γ pretreated colon 26 cells were resistant to lysis when incubated in concentrations of HBSS greater than 25%. At lower concentrations, the percentage lysis increased steeply but the differences in the osmotic fragility with IFN-γ and control pretreatment were not significant (Table 6).

**Susceptibility of colon 26 cells to TNF-α.**

Growth inhibition of colon 26 cells was investigated using MTT assay described in Materials and Methods. Table 7 demonstrates that colon 26 cells pretreated by IFN-γ obtain resistance to TNF-α mediated growth inhibition.

**Effect of cycloheximide on IFN-γ induced enhancement of metastasis.** Colon 26 cells were pretreated with IFN-γ (100 IU/ml) and cycloheximide (50 μg/ml) for 1 h and metastatic ability was investigated. There was the enhancement of metastases by pretreatment with IFN-γ alone, while there was no longer difference in metastatic ability between control and IFN-γ pretreatment in the presence of cycloheximide (Table 8). The metastatic ability of non-IFN-γ-pretreated tumor cells was unaffected by the treatment with cycloheximide.

**Discussion**

Metastasis formation is a complex process involving several sequential steps that tumor cells have to complete after their release from the primary tumor (14). Physiological systemic or local alterations can influence the tumor cells’ ability to disseminate and metastasize. Morphological studies on the intravascular arrest of circulating tumor cells indicate an initial arrest of tumor cells characterized by a tumor-endothelial cell contact during the first hour, followed by penetration of the tumor cells through the vascular endothelium after 4 h and their complete extravasation by about 24 h (15). The organ distribution and arrest of radiolabeled tumor cells have been often used as an experimental tool for following the dissemination of tumor cells. Our data show that no difference in the accumulation of tumor cells in the lung was observed between the control and IFN-γ groups in the first 4 h and the loss of cell-associated cpm from the lungs was rapid between 4 and 24 h as described (11), but at 24 h the retention of IFN-γ-treated colon 26 cells was significantly higher in the lungs. This distribution pattern suggests that IFN-γ does not interfere with the ability of

---

**Table 5** Effect of IFN-γ on susceptibility to lymphokine-activated killer (LAK) cell-activated lysis

<table>
<thead>
<tr>
<th>Pretreatment of tumor cells</th>
<th>Effector: Target</th>
<th>50:1</th>
<th>100:1</th>
<th>200:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td></td>
<td>5.9†</td>
<td>9.0</td>
<td>12.6</td>
</tr>
<tr>
<td>IFN-γ×</td>
<td></td>
<td>4.9</td>
<td>6.4</td>
<td>8.2</td>
</tr>
</tbody>
</table>

† LAK cells used in this experiment caused 44.9% specific lysis of YAC-1 target cells at a effector/target ratio of 100:1.
× Data represent % specific lysis.
× Colon 26 cells were pretreated with 100 IU/ml IFN-γ for 1 h. IFN-γ: spilled out in Table 1.

**Table 6** Effect of IFN-γ on osmotic fragility

<table>
<thead>
<tr>
<th>Pretreatment of tumor cells</th>
<th>% of HBSS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Medium</td>
<td>18.1†</td>
</tr>
<tr>
<td>IFN-γ×</td>
<td>17.2</td>
</tr>
</tbody>
</table>

† Data represent % specific lysis.
× Colon 26 cells were pretreated with 100 IU/ml IFN-γ. IFN-γ: spilled out in Table 1.

**Table 7** Susceptibility of colon 26 cells to tumor necrosis factor α (TNF-α)

<table>
<thead>
<tr>
<th>Pretreatment of tumor cells</th>
<th>TNF-α (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10²</td>
</tr>
<tr>
<td>Medium</td>
<td>24.8†</td>
</tr>
<tr>
<td>IFN-γ×</td>
<td>0.7</td>
</tr>
</tbody>
</table>

† Data represent % growth inhibition.
× Colon 26 cells were pretreated with 100 IU/ml of IFN-γ for 1 h.

**Table 8** Effect of cycloheximide on IFN-γ induced enhancement of metastasis

<table>
<thead>
<tr>
<th>Treatment of tumor cells</th>
<th>Pretreatment of tumor cells</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medium</td>
<td>IFN-γ×</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>13.4 ± 6.3†</td>
</tr>
<tr>
<td>Cycloheximide××</td>
<td>12.6 ± 4.2</td>
<td>14.6 ± 4.9</td>
</tr>
</tbody>
</table>

† Colon 26 cells were pretreated with 100 IU/ml IFN-γ for 1 h. IFN-γ: spilled out in Table 1.
× Data represent number of lung metastases. Values are the mean ± SD of five mice.
× Colon 26 cells were incubated with 50 μg/ml of cycloheximide for 1 h before IFN-γ pretreatment.
* P < 0.01 versus medium control.
tumor cells to become trapped in the lung, but with their clearance in the secondary organ.

One possible mechanism of the reduced clearance of the trapped colon 26 tumor cells is that the pretreatment with IFN-γ could stabilize the cell membrane of tumor cells. However, this is unlikely in this system, since osmotic fragility was unaffected by IFN-γ pretreatment. Alternatively, IFN-γ-pretreated tumor cells may be resistant to the elimination mediated by host cells resulting in the reduction in clearance in the lung. We demonstrated that, in normal immunocompetent mice, pretreatment of colon 26 cells with IFN-γ significantly increased the number of metastatic loci in the lung, and that, in anti-AGM-Ab-treated mice, pretreatment of tumor cells with IFN-γ did not produce any further enhancement of metastasis as compared with the control cells.

These in vivo experiments suggest that enhancement of metastases by IFN-γ is due to the increasing resistance of colon 26 cells to asialo GM1-positive cells. Previous reports have suggested that in vitro treatment of tumor cells with IFN-γ leads to a decrease in their susceptibility to NK cells (16, 17). Our results show that colon 26 cells were completely resistant to lysis by NK cells. It is possible that populations of asialo GM1-positive cells other than NK cells such as macrophages may mediate antineoplastic activity (18). Consistent with this idea, our data suggest that colon 26 cells pretreated with IFN-γ acquired resistance to growth inhibition mediated by TNF-α since which is a major effector molecule for tumor cell destruction produced by macrophages (19).

The acquisition of resistance against TNF α-mediated cytotoxicity is assumed to depend on certain protective proteins involved directly or indirectly. These include lysosomal enzymes (20), manganous superoxide dismutase (21), and Bel-2 protein (22). The effects of IFN-γ on protein induction have been studied by many investigators. A variety of enzymes, cell surface antigens, receptors, adhesion molecules, and a number of proteins of unknown function were shown to be induced by IFN-γ (23). Our results suggest that the enhancement of metastases by IFN-γ was dependent on de novo protein synthesis since the enhancement was abolished in the presence of cycloheximide. Theoretically, IFN-γ may enhance metastatic ability by modulating any one (or more than one) of these.

There is no doubt that IFN-γ has useful antitumor activities in animal models and some human malignancies. IFN-γ has been used in clinical trials in patients with hematologic malignancies and solid tumors such as malignant melanoma, renal cell carcinoma and colon carcinoma. No enhancement of metastases has been reported in these clinical trials, although the efficacy of IFN-γ as an antitumor agent was not so much as that used in animal models (24). In a clinical trial of IFN-γ as an adjuvant in high-risk stage I and II cutaneous malignant melanoma, the number of relapses or deaths and the median time to relapse in patients with stage II disease were found to be worse in the IFN-γ group than in nontreatment group (25). However, it may be difficult to accurately assess the enhancement of metastasis in such a clinical trial. Studies such as those reported here suggest that IFN-γ could, in some circumstances, have deleterious actions in the complex tumor-host relationships. Consequently, IFN-γ should be handled with care for the treatment of patients with malignant diseases. Additional studies are required to determine the precise mechanism by which IFN-γ enhances experimental metastasis and will lead to improved treatment of advanced cancer.

References

10. Corbett TH, Griswold DP Jr, Roberts BJ, Peckham JC and Schabe FM Jr: Tumor induction relationships in development of transplantable...
cancers of the colon in mice for chemotherapy assays, with a note on
11. Fidler IJ: Metastasis: Quantitative analysis of distribution and fate of
tumor emboli labeled with 125I-iodo-2-deoxyuridine. J Natl Cancer
12. Weiss L and Clement K: Studies on cell deformability: Some
DL, Abbott BJ, Mayo JG, Shoemaker RH and Boyd MR: Feasibility of
drug screening with panels of human tumor cell lines using a mi-
(Lond.) (1980) 283, 139-146.
15. Crissman JD, Hatfield JS, Menter DG, Siegel B and Horn KV:
Morphological study of the interaction of intravascular tumor cells with
endothelial cells and subendothelial matrix. Cancer Res (1988) 48,
4065-4072.
16. Welsh RM, Karre K, Hansson M, Kunkel LA and Kiessling RW:
Interferon-mediated protection of normal and tumor target cells
against lysis by mouse natural killer cells. J Immunol (1981) 126,
219-225.
17. Gronberg A, Fornal MT and Ortaldo JR: IFN-γ treatment of K562 cells
inhibits natural killer cell triggering and decreases the susceptibility
to lysis by cytoplasmic granules from large granular lymphocytes. J
protection of B16 melanoma cells from cytotoxicity by activated
19. Urban JL, Shepard HM, Rothstein JL, Sugarman BJ and Schreiber H:
Tumor necrosis factor: A potent effector molecule for tumor cell killing
by activated macrophages. Proc Natl Acad Sci USA (1986) 83, 5233-
5237.
20. Liddil JD, Dorr RT and Succhini P: Association of lysosomal activity
with sensitivity and resistance to tumor necrosis factor in murine L929
21. Wong GHW and Goeddel DV: Induction of mammalian superoxide
dismutase by tumor necrosis factor: Possible protective mechanism.
22. Hermet T, Bentor J, Richter C and Peterhans E: Expression of
BCL-2 protein enhances the survival of mouse fibrosarcoma cells in
1456-1460.
induction in human fibroblasts in response to treatment with interferon-
α, interferon-γ, interleukin 1α, interleukin 1β, and tumor necrosis
25. Meyers PA, Kopecky K, Samson M, Hersh E, MacDonald J, Jaffe H,
Crowley J and Colman C: Recombinant human interferon-γ: Adverse
effects in high-risk Stage I and II cutaneous malignant melanoma. J
Natl Cancer Inst (1990) 82, 1071.

Received February 10, 1995; accepted November 27, 1995.