Interferon Gamma is Highly Effective Against Orthotopically-Implanted Human Pleural Adenocarcinoma in Nude Mice

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Abstract. The efficacy of recombinant human gamma interferon (rh IFN-\(\gamma\)) was evaluated for the treatment of human pleural adenocarcinoma in a patient-like nude mouse model which is constructed by surgical orthotopic implantation (SOI) of histologically-intact human tumor tissue. The human non-small-cell lung cancer cell line H-460 was used for the study. Gamma interferon was tested in three different dosages (25,000 U, 50,000 U and 100,000 U) versus an untreated control through i.p. injection twice a day for five days, which was started 48 hours after SOI; The results showed that IFN-\(\gamma\) can prolong the survival time of the tumor-bearing animals. The symptoms and signs of hypoxia such as restricted physical activity and cyanosis due to primary tumor growth in the thoracic cavity as well as cachexia developed much earlier in the control than in the IFN-\(\gamma\)-treated mice. The mice in the control group had succumbed by day-23 after tumor implantation, however at that time 67% of the mice in the 100,000 U-treated group, 15% of the mice in the 50,000 U-treated group, and 16% of the mice in the 25,000 U-treated group were still alive. The orthotopically-transplanted tumor grew rapidly and metastasized to the lung and liver in the untreated control. In the IFN-\(\gamma\)-treated groups both primary tumor growth and metastasis were reduced, probably accounting for the increased survival rate. The results demonstrated dose-dependent efficacy of IFN-\(\gamma\) in suppressing symptomology, primary tumor growth, invasiveness and metastasis of the human lung cancer cell line H-460, and increased survival of the tumor-bearing animals. These results suggest clinical trials of IFN-\(\gamma\) should begin for treatment of pleural adenocarcinoma for which there is no current effective therapy.

The median survival time of patients with adenocarcinoma of the pleura usually ranges between 6 and 12 months (1). Intensive treatment regimens with chemotherapy, radiotherapy or surgery are not satisfactory and have little long-term effect (2). Thus effective treatment for this deadly disease needs to be developed.

Immunotherapy, as a new modality for the treatment of refractory malignant diseases, has been investigated preclinically and clinically. Gamma interferon is a lymphokine produced by T-lymphocytes in response to specific antigenic or tumorigenic stimuli, which has immunomodulatory and direct antitumor properties. It has been reported that the antiproliferative effect of IFN-\(\gamma\) is 10-100 fold greater than that of IFN-\(\alpha\) or IFN-\(\beta\) (3), suggesting a greater potential for immunotherapy of malignant diseases. Recombinant DNA technology made it possible to produce many lymphokines in large quantities including IFN-\(\gamma\), which has enabled evaluation of the immunomodulatory and therapeutic activities of these lymphokines. As a new immunotherapeutic agent, IFN-\(\gamma\) has been intensively studied in both animal experiments and clinical trials as a single agent or combined with other lymphokines, such as IL-2, IFN-\(\alpha\) and TNF, or with chemotherapeutic agents (4-8). Although a large body of data has been obtained, as noted by Aultsky et al (9), this treatment modality should be further tested in a variety of disease states, with optimal dosage, route and schedule to be determined.

In this study, we tested the efficacy of rh IFN-\(\gamma\) in the treatment of human pleural adenocarcinoma at three
Table I. IFN-γ treatment of nude mice with orthotopically-implanted human pleural adenocarcinoma.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Number of mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Untreated control</td>
<td>5</td>
</tr>
<tr>
<td>Group 2</td>
<td>IFN-γ 25,000U</td>
<td>5</td>
</tr>
<tr>
<td>Group 3</td>
<td>IFN-γ 50,000U</td>
<td>5</td>
</tr>
<tr>
<td>Group 4</td>
<td>IFN-γ 100,000U</td>
<td>5</td>
</tr>
</tbody>
</table>

Table II. The effect of IFN-γ on the cancer symptomology of nude mice with orthotopically-implanted human pleural adenocarcinoma.

<table>
<thead>
<tr>
<th>Group</th>
<th>*Time of onset of cyanosis and restricted physical activity</th>
<th>*Time of onset of body weight loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Group 1)</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>IFN-γ 25,000U (Group 2)</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>IFN-γ 50,000U (Group 3)</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>IFN-γ 100,000U (Group 4)</td>
<td>20</td>
<td>22</td>
</tr>
</tbody>
</table>

*The time of onset refers to days after tumor implantation.

Human recombinant IFN-γ was administrated by i.p. injection twice a day for five days.

from the incision in the 4th intercostal space. Ten pieces of tumor tissue were sutured onto the suture and then pushed into the pleural cavity. The needle was then inserted into the cavity again and pulled out through the 3rd intercostal space. The suture was knotted across the incision to close the opened pleural cavity. Pneumothorax was relieved by removing air from the pleural cavity using a sterile 3cc syringe with an attached 25G 5/8 gauge needle right after the pleural cavity was closed. The skin and muscle layers of the thorax were then closed using a 6-0 surgical suture (Look Incorporated, Norwell, MA). Mice resumed their activity soon after isoflurane inhalation was discontinued.

Experimental design. Twenty mice were randomly assigned to the 4 groups designated in Table I the second day after surgery. IFN-γ was administered by i.p. injection twice a day for 5 days. The administration started 48 hours after surgery. All mice were under close observation for any abnormal symptoms and signs. All dead mice were immediately immersed in formalin for subsequent autopsy. Survival time of the mice primary tumor measurement after necropsy as well as pathological analysis of the primary tumor and metastases were determined in all mice. Histology study was performed by standard HE staining.

Results and Discussion

Tumor development and symptomology. Visible chest wall invasion, the symptoms and signs of hypoxia such as restricted physical activity and cyanosis, and cachexia with evident body weight loss appearing earliest in the control group and latest in the highest-dose IFN-γ-treated group (Table II). These symptoms and signs developed in the tumor-implanted mice represent one of the patient-like aspects of the SOI model.

Tumor size and weight. Tumors were resected and measured by the two largest diameters with calipers and then weighed.
using an electronic balance during autopsy. Differences between groups at autopsy with statistical significance could not be obtained. See Table III for numerical data.

Findings of invasion and metastasis at autopsy. Gross autopsy demonstrated that the degree of tumor spread and invasiveness decreased in the order of increasing IFN-γ dose. Two mice in the control group were found with left lung and liver metastasis, one mouse in the 25,000-unit-treated group
had liver metastasis, while no mice had metastatic lesions in the 50,000-unit-treated and 100,000 unit-treated groups. The metastatic lesions were confirmed by histology study (Figure 1). Histological examination of primary tumors also displayed more regional necrosis in the highest-dose group, indicating efficacy.

Survival rate. The average survival time of the IFN-γ-treated groups (Group 2 treated with IFN-γ 25,000 units: 21.2 days, Group 3 treated with IFN-γ 50,000 units: 21 days and Group 4 treated with IFN-γ 100,000 units: 26.4 days) were longer than the control group (Group 1: 19 days), which, when paired one by one, had statistical difference (p<0.05 for Group 1: Group 2 and 3; p<0.01 for Group 1: Group 4, Log Rank Test). Although there was no statistical difference between Group 2 and Group 3, a difference was seen when these two groups were compared with Group 4 (p<0.01).

Systemic treatment of solid tumors, including pleural adenocarcinoma, with conventional methods has been generally very disappointing. Gamma interferon can influence, both directly and indirectly, several aspects of immunoreactivity (23–25), such as augmentation of cytotoxicity mediated by NK cells (26) and macrophages (27), so as to enhance the pre-existing antitumor mechanisms in the tumor-bearing host. Therefore much attention has been devoted to this biological modifier for its application in the treatment of malignant diseases. Nevertheless different results regarding the dosage, route and schedule of the administration as well as toxicity have been reported. Talmadge et al (28) reported that IFN-γ activated natural

<table>
<thead>
<tr>
<th>Group</th>
<th>Primary tumor weight at autopsy (gram)</th>
<th>Survival time (days)</th>
<th>Incidence of invasion (1) and metastasis (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>(1)**</td>
</tr>
<tr>
<td>Control (Group 1)</td>
<td>2.94 ± 0.73</td>
<td>19.0 ± 3.16</td>
<td>5/5</td>
</tr>
<tr>
<td>IFN-γ 25,000U (Group 2)</td>
<td>2.14 ± 0.63</td>
<td>21.2 ± 3.63</td>
<td>3/5</td>
</tr>
<tr>
<td>IFN-γ 50,000U (Group 3)</td>
<td>2.40 ± 0.42</td>
<td>21.0 ± 3.16</td>
<td>1/5</td>
</tr>
<tr>
<td>IFN-γ 100,000U (Group 4)</td>
<td>2.38 ± 0.44</td>
<td>26.4 ± 3.97</td>
<td>0/5</td>
</tr>
</tbody>
</table>

* p<0.05 between Group 1 and Group 2; Group 1 and Group 3. * p>0.05 between Group 2 and Group 3
* p<0.01 when Group 4 was compared with Group 1, Group 2 and Group 3.
** The sites of invasion counted included right hilus and right pleural cavity.
*** The metastatic sites included lung and liver.

Human recombinant IFN-γ was administered by i.p. injection twice a day for five days.
killer cells and macrophages in a dose-dependent manner in vivo, manifesting a bell-shaped therapeutic response curve. Optimal activity was observed after i.v. administration of 50,000 U/mice three times per week. They also stressed that intravenous administration of IFN-γ resulted in significantly greater therapeutic activity than i.m. or i.p. routes of administration. However, in a study investigating the synergism of IFN-γ with TNF and IL-2 against a natural killer-resistant pulmonary metastasis of the MCA sarcoma, Agah et al. (4) found that i.p. administration of up to 20,000 U/mouse twice daily for 5 days was not efficacious.

In our study, we tested the efficacy of rh IFN-γ in three different doses by i.p. route in a patient-like nude mouse model of human pleural adenocarcinoma. The results showed that the highest efficacy was demonstrated at the highest dose (100,000 U/animal) tested. Less efficacy was seen in the two lower dosages (50,000 U/animal and 25,000 U/animal) tested with no statistical difference between these two dosages, which might suggest that different doses within a certain range might not be able to generate different biological effects.

The cell line H460, when transplanted by SOL, is a very aggressive and fast-growing tumor. In this study the untreated animals implanted with SOL had only an average survival time of 19 days. Mice constructed by SOL with this cell line also demonstrate symptoms and signs of hypoxia, which can not be observed in a.s.c. model. This is the first study of the H460 line when transplanted by SOL and it revealed that the high primary orthotopic growth and metastatic potential of this tumor.

Almost all the mice in the four groups eventually succumbed to severe hypoxia, which was probably caused mainly by the primary tumor burden in the thoracic cavity. Examination during autopsy revealed that the mediastinum was pushed to the right side, and the whole left pleural cavity was occupied by the primary tumor in all the 20 mice autopsied. Although the size and weight of the primary tumor measured at autopsy was similar in all groups with no statistical difference, observations during the course of the experiment showed that mice in the control group demonstrated hypoxic symptoms and signs such as restricted physical activity and cyanosis as early as 8 days after orthotopic tumor implantation. On the other hand, whereas mice treated with the highest dose of IFN-γ showed these symptoms and signs after 20 days of tumor implantation (Table II), All the IFN-γ-treated mice survived statistically significantly longer than the untreated control (Figure 2). This indicates that the primary tumor grew slower in the longer-lived IFN-γ-treated animals, even though the primary tumor sizes in all groups were similar at autopsy. The survival curve showed that when all the mice in the control group died, still 67% of the mice in Group 4 (treated with IFN-γ 100,000U), 15% in Group 3 (treated with IFN-γ 50,000U) and 16% in Group 2 (treated with IFN-γ 25,000U) remained alive (P<0.05) (See Figure 2).

Agah et al (4) noted that a low and nontoxic dose (10,000 U/animal) of the lymphokines IFN-γ, TNF or IL-2 tested alone have no antitumor effect against disseminated neoplasia. Tamadge et al (29) described, in another report on the therapeutic properties of IL-2 for the treatment of experimental metastasis, that significant augmentation of splenic NK cell activity was observed at doses of 50,000 U/animal and maximal activity was observed at doses of greater than 100,000 U/animal. The results reported here demonstrated that the highest dose generated the highest anti-metastatic effect as shown in Table III.

As reported by Aulitzky et al (9) in a clinical trial for the treatment of metastatic renal cell carcinoma with IFN-γ, the main side effects of IFN-γ in patients were fever, fatigue and chills. There were other adverse effects like nausea, vomiting, anemia and pruritus, which were less frequently encountered. In our study reported here there were difficulties in monitoring the above side effects in mice. However, close observation during the experiment did not reveal any treatment-related abnormalities. All mice died of the effects of the tumor.

Clinical trials have been performed to test the efficacy of IFN-γ in other malignant diseases such as renal cell carcinoma (30), ovarian carcinoma (31, 32), melanoma (33, 34), malignant mesothelioma (35), squamous cell carcinoma (36), hepatocellular carcinoma (37), breast carcinoma (38), as well as recent studies on small-cell lung cancer (39, 40). However, an optimal treatment regimen is far from determined. In addition, different diseases and disease states might require different regimens for obtaining the maximum therapeutic effect and minimum toxicity. Many authors reached the conclusion that a biologically active dose (BAD) might be an optimal choice and more effective than the maximum-tolerated dose (MTD), such as was shown for natural killer activity and macrophage cytotoxicity (26, 33, 34, 41, 42). In our study reported here, we demonstrated good tolerance and efficacy of rh IFN-γ at 100,000 units, which did not necessarily mean there might not exist other better dosing regimens, since higher doses or doses less than 25,000 units were not tested in this study. Nevertheless, our results are encouraging and provides a basis for further studies and for examining whether this might be a valuable therapeutic regimen in patients with pleural adenocarcinoma.

References


41 Kleinerman ES and Kurzrock R et al: Activation or suppression of the
tumoricidal properties of monocytes from cancer patients following
activity with human recombinant gamma-interferon. Cancer Res
42 Weiner CM and Steplewski Z et al: Divergent dose-related effects of
gamma-interferon therapy on in vitro antibody-dependent cellular
and non-specific cytotoxicity by human peripheral blood monocytes.
43 Wang X, Fu X and Hoffman RM: A new patient-like metastatic
model of human lung cancer constructed orthotopically with intact
tissue via thoracotomy in immunodeficient mice. Int J Cancer 51: 992-
44 Astoul P, Colt HG, Wang X and Hoffman RM: Metastatic human
pleural ovarian cancer model constructed by orthotopic implantation
of fresh histologically-intact patient carcinoma in nude mice.
45 Astoul P, Wang X and Hoffman RM: “Patient-like” nude and SCID
mouse models of human lung and pleural cancer. Int J Oncology 3:
46 Wang X, Fu X and Hoffman RM: Matrix metalloproteinase BB-94
(Batimastat) inhibits human colon tumor growth and spread in a
patient-like orthotopic model in nude mice. Cancer Res 54: 4726-

Received March 6, 1996
Accepted April 22, 1996