Failure of Interferon to Inhibit Plutonium-Induced Osteosarcomas in Mice


ABSTRACT—The injection of murine interferon three times weekly in dose levels of 1,250, 5,000, or 20,000 U produced no significant antitumor effect against primary 239Pu-induced osteosarcomas in C57BL/6J mice. The interferon treatment was begun 94 days after the plutonium injection, which is well before the radiographic or microscopic appearance of neoplasia, and was continued until the moribund state or death. The average radiation dose accumulated by the skeleton at the time of first treatment was approximately 300 rad. The largest dose of interferon studied, 20,000 U/injection, was approximately 3×10^6 U/m^2 of body surface, or 10^6 U/kg body weight.—JNCI 1984; 72:1137-1140.

The interferons, discovered in 1957 by Isaacs and Lindenmann (1), are antiviral proteins produced in humans and in several different classes of lower animals, including teleost fish (2, 3). Their production is initiated by some viruses, but other natural and synthetic agents have also been shown to stimulate their production, especially double-stranded RNA (4-6). Interferons constitute one of the first lines of defense against viruses and are most active when derived from cells of the same species, but cross-reactivity has been observed within animals of the same order or family and even between rabbit and humans (6).

The antiviral action of interferons is well established and was the basis of their discovery and their name (1, 7). However, more recently, other actions have been demonstrated, such as increased cytotoxicity of double-stranded RNA, enhancement of macrophage and natural killer cell activity, effects on the immune response, and suppression of the growth of both normal and malignant cells (8-17). The inhibition of osteosarcoma metastases in humans and bone tumor implants in experimental animals has been of special interest to those concerned with the toxicity of radium, nuclear fuel such as plutonium, and nuclear power waste products such as the actinide elements, americium, and curium, because radiation-induced bone cancer is one of the major endpoints resulting from internal burdens of these elements (18).

MATERIALS AND METHODS

All of the animals used in the radiation phase of this study were C57BL/6J mice, which were maintained in a temperature-controlled environment [21±3°C (SD)]. Lighting was regulated at 12 hours each of light and darkness.

The radionuclide (2.75 μCi 239Pu/kg) was administered in a citric acid-sodium citrate buffer (pH 3.5 and 0.08 M in total citrate) via a single ip injection of 0.2 ml at 87 days of age.

To achieve a high tumor incidence, we used females only, since it is a well-established fact that the female mouse is about four times more sensitive than the male mouse to radiation-induced bone cancer (19).

A postmortem examination was conducted on each animal and included a histologic study of all gross lesions and of lungs, liver, kidneys, spleen, and vertebrae. Tumor diagnosis was based initially on radiographs with subsequent histologic confirmation. Radiographs were taken only after death or in the moribund state, several days preceding death, so that the radiation dose from X-rays was not an etiologic factor in any of the tumors.

The absence of any effect of the interferon treatment on the retention of plutonium and thus the radiation dose was demonstrated by a comparison of the radionuclide burden in interferon-treated and untreated animals that had received the same amount of plutonium injections. In most instances this comparison was based on the activity in the paired femora, although some radionuclide assays of the entire skeleton were made.

The partially purified, murine type I interferon preparation utilized in this study was produced in L-cells infected with Newcastle disease virus and furnished by Dr. Kurt Paucker, Department of Microbiology, The Medical College of Pennsylvania and Hospital, Philadelphia, Pa., through the National Cancer Institute. The lyophilized interferon was received as two different lots and stored at −70°C until used. According to Dr. Paucker’s directions, each lot of interferon was constituted with PBS as follows: lot #2642 at 5.6×10^6 U/ml (sp act, 4.0×10^7 U/mg protein) and lot #2750 at 9.0×10^6 U/ml (sp act, 7.3×10^7 U/mg protein). The titers provided for these preparations were confirmed in our laboratory by the 50% plaque reduction assay in L-929 cells.

ABBREVIATION USED: PBS=phosphate-buffered saline.

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2 Supported by U.S. Department of Energy contract DE-AC02-76EV-00119 and Public Health Service grant CA-23475 from the National Cancer Institute.
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cells challenged with vesicular stomatitis virus. The antitumor and antiviral activities of these preparations in mice have been reported previously (8, 10, 20, 21). Reconstituted interferon was further diluted with PBS to the desired number of units per milliliter for injection into mice. Mice were given 20,000, 5,000, or 1,250 U interferon in 0.1 ml volume sc in the ventral abdominal area three times weekly (Monday, Wednesday, and Friday), beginning 94 days after 239Pu injection and continuing until death or the moribund state. Control mice received 0.1 ml PBS sc at the same site and on the same days as the interferon-treated mice. At the time the interferon treatment was initiated, the average skeletal radiation dose was approximately 300 rad; however, at this time, the bone tumors were still in the latent state and could not be found by radiologic or histologic examination.

RESULTS

Of the 133 plutonium-injected mice observed in this study, 89 developed 1 or more bone tumors. Most of the neoplasms occurred in the axial skeleton and principally in the vertebrae. Histologically, they were all osteosarcomas and generally were very osteogenic. The distribution of these tumors within the various treatment groups is summarized in table 1. A chi-square analysis of the tumor incidence data did not indicate a significant difference between the interferon-treated and PBS-treated mice (5 < P < 7). Also, the difference in the survival times of the respective groups was not significant.

A comparison of the last two columns of table 1 indicates the presence of significant plutonium-induced competing risks other than bone cancer. This is especially obvious when the respective survival times are compared with an average mean life expectancy of 789 days for the female C57Bl/6j mouse (22) and of 811 days for our laboratory's female C57Bl/Do mice of the Dougherty strain (19). The major radiation-induced, life-shortening factor, other than bone cancer, was the induction of hematopoietic disorders, especially a very high incidence of aplastic anemia. The latter was associated with marked extramedullary hematopoiesis in the liver and spleen of some of the mice. This occurred in both the control and treated groups and appeared to be a radiation-induced effect and not an interferon-induced effect, although bone marrow suppression is reported to be a side effect of exogenously administered interferon (23). The amount of life shortening associated with causes other than osteosarcomas was not significantly different than that produced by the bone cancers. The interferon therapy did not change this relationship.

The cumulative bone tumor incidence rate is shown in text-figure 1. The data depicted in the respective curves indicate the following:

1) The earliest tumor deaths occurred among the mice receiving interferon treatment.
2) The earliest tumor death in the 20,000-U treatment group, the highest dose level of interferon, was similar to the earliest tumor death in the mice receiving no interferon.
3) Fifty percent of the mice in all groups, including the controls, had died of bone cancer by 390-425 days post injection.
4) The temporal factors were similar in all dose levels, with the possible exception of the intermediate dose (5,000 U interferon/injection). The slope of the curve depicting this dose level was more flat in its terminal aspect, tending to deviate from the pattern of the other three treatment regimens. However, this possibly did not indicate an antitumor effect related to the interferon treatment for the following reasons: a) The curves, as presented, reflect the time from injection to death and not tumor latency. This is significant, since part of the tumors associated with long survival times in this dose level, possibly due to chance, arose in anatomical sites that allowed the mouse to remain ambulatory for longer periods after the onset of neoplasia. b) This dose level ultimately resulted in the highest incidence of bone cancer of any of the groups (table 1).
5) The short initial times to death with tumors in the mice receiving 1,250 U are unexplained, but they could be due to chance: We consider it unlikely that the lower dosage would shorten the minimal latent period without a similar effect at higher dosages.
6) A dose-related effect, such as a progressive relationship between the amount of interferon administered and the incidence of osteosarcomas, was not apparent.
7) The group receiving the highest dose of interferon had the lowest bone tumor incidence (55%), but this was not a statistically significant difference and the survival time of this group was not increased over that of mice receiving no interferon.

Table 1.—Comparison of survival time, osteosarcoma incidence, and tumor latency in mice receiving various dose levels of murine interferon.

<table>
<thead>
<tr>
<th>Units of interferon</th>
<th>No. of mice</th>
<th>No. (and % ± SD) with osteosarcoma</th>
<th>Average days post injection to death with bone cancer ± SD</th>
<th>Average days post injection for mice dying without bone cancer ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>42</td>
<td>28 (67±7)</td>
<td>365±35</td>
<td>375±32</td>
</tr>
<tr>
<td>20,000</td>
<td>31</td>
<td>17 (55±9)</td>
<td>366±30</td>
<td>360±55</td>
</tr>
<tr>
<td>5,000</td>
<td>30</td>
<td>22 (77±8)</td>
<td>407±78</td>
<td>331±68</td>
</tr>
<tr>
<td>1,250</td>
<td>30</td>
<td>21 (70±8)</td>
<td>356±63</td>
<td>354±68</td>
</tr>
</tbody>
</table>

*All mice were given a single ip injection of 2.75 μCi 239Pu/kg at 87 days of age.

†Given sc triweekly, starting 94 days after 239Pu injection.
**DISCUSSION**

The inhibition of mouse osteosarcoma cells by interferon has been established by tumor implant studies in mice and also in in vitro experiments (8, 17). Thus the ineffectiveness against primary radiation-induced bone sarcoma observed in this experiment was unexpected, especially since the interferon was administered three times weekly and considerably in advance of any microscopic or radiographic evidence of neoplasia. Also, the developing neoplasms were probably exposed to injected interferon while in the 1-, 2-, or 4-cell stage, an ideal situation with respect to chemotherapy.

The most obvious difference between this experiment involving primary tumors and the more successful in vitro or transplant studies was the local concentration of interferon achievable at the tumor target site. For example, in one of the transplant experiments, inhibition was noted when an injection of osteosarcoma cells into the subcutis was followed immediately by twice daily injections of 30,000 U interferon per injection into the area of tumor implantation for 7 days (10). Partial success was again noted with experimentally induced osteosarcoma lung metastases when daily iv injections were administered during the 1st week after implantation (8). Also, interferon ip administered inhibited the growth of tumors implanted into the peritoneal cavity (25). Each of these treatment procedures establishes a relatively high local concentration of interferon at the tumor site. Even higher levels can be achieved with in vitro techniques, which produce the most significant growth inhibition.

In contrast to these somewhat artificial conditions, the experiment presented in this paper evaluated the effect of a general systemic level resulting from an sc injection of interferon far from the target area, which was usually the endosteal surface of bone trabeculae in the skeleton. The potential tumor sites were further isolated by a relatively low blood supply, estimated to be approximately 18–20 ml/100 g/minute, although significant variation in the vascularity has been demonstrated in various regions of the skeleton or in the same bone in some species. For example, in the canine femur, the flow rate varies from approximately 5 cm³/100 g bone/minute in the mid-diaphysis to a high of 27 cm³/100 g of bone/minute in the femoral neck (26, 27). This range is appreciably less than the supply to some of the other tissues such as the lung.

The obvious solution to the problem of achieving a higher concentration at the tumor site is the use of much larger doses. However, there is no evidence that neoplastic tissues are any more sensitive to interferon inhibition than normal cells (14), which suggests that toxicity, especially in rapidly dividing tissues, will ultimately occur with escalating systemic doses, a problem that arises in most forms of chemotherapy. Thus one of the unanswered questions is: Can the general systemic concentration of interferon be raised to an antitumor level without serious toxicity? Obviously, the injected dose or the frequency of administration used in this study was too low or infrequent for suppression of primary radiation-induced osteosarcomas in mice.

In summary, these data indicated that graded doses of
concentrations at the tumor site rather than to insensitivity of this type of neoplasia to interferon. This seemed probable inasmuch as the same interferon inhibited mouse osteosarcoma cells in vitro.

REFERENCES