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Antitumor Effect of Human Leukocyte Interferon on Human Osteosarcoma Transplanted into Nude Mice*

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Abstract—*We studied the effect of human leukocyte interferon (HuIFN-α) on a human osteosarcoma (OS-OH) transplanted and passed serially in athymic mice. The growth of OS-OH was strikingly inhibited by HuIFN-α* (50,000 IU/*mouse), regardless of whether the interferon treatment was initiated* 24 hr *after tumor inoculation or* 2 weeks *later, when tumors had grown to an appreciable size* (4–6 mm). *The antitumor effect of HuIFN-α was found to be dose-dependent and a daily administration of HuIFN-α* (50,000 IU/*mouse) all but completely arrested the tumor growth.*

INTRODUCTION

ISAACS and Lindenmann found in 1957 that the allantoic fluid of eggs exposed to irradiated virus possessed an interfering capacity which was distinct from the virus [1] and the substance responsible was named 'interferon'. Since then, numerous studies on interferon (IFN) have been carried out in *in vitro* and *in vivo* systems. In particular, the antitumor effect of IFN has been reported by Gresser, Cantell, Strander and others [2–9]. We carried out studies on the antitumor effects of human leukocyte interferon (HuIFN-α) on a human osteosarcoma transplanted and passed serially in athymic mice and our findings are reported herein.

MATERIALS AND METHODS

Mice

Animals used were 5–9-week-old female nude mice with an BALB/c genetic background and which were bred at the Central Institute for Experimental Animals, Kawasaki, Japan. All mice were kept in a flexible vinyl film isolator and bedding, cages, food and water were autoclaved before being brought into contact with the mice [10, 11]. Daily observations were recorded and autopsy was done on all the mice.

Tumor

The tumor from which we derived line OS-OH were histologically diagnosed as osteosarcoma from a pulmonary metastasis of an osteosarcoma of the right femur of a 21-year-old Japanese student. Histologic appearances of the serially transplanted OS-OH were identical to those of the original tumor (Fig. 1). At the time of the study the tumor was between the 4th and 9th passages in nude mice.

Human leukocyte interferon (HuIFN-α)

Crude leukocyte interferon was induced from human leukocyte suspensions inoculated with Sendai virus. The interferon used in those experiments was purified by chromatography on a CM-cellulose column and gel-filtration on a Sephadex G-50 column. The interferon, obtained from The Green Cross Co., Osaka, Japan [12] in the form of lyophilized interferon (1.0×10^6 IU/vial), was reconstituted by dissolving in 10 ml of sterile 0.9% NaCl, resulting in a concentration of 1.0×10^5 IU/ml. The specific activity was approximately 50,000 IU/mg protein. Interferon was administered i.p. and control mice were given 0.5 ml of 0.9% NaCl solution.

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Interferon assay

Interferon activities were assayed using a plaque reduction method consisting of human amniotic cells (FL cell) and vesicular stomatitis virus. The reciprocal of the dilution which reduced the plaque by 50% was defined as the titer [12]. Interferon titers are expressed in terms of reference standard B, 69/19 for human interferon (Medical Research Council, National Institute for Biological Standards and Control, London, U.K.).

Tumor inoculation

Solid tumor blocks of about 2 mm in diameter were inoculated with a trocar into the subcutaneous space on the flank and the tumor take was 100%. Spontaneous regression did not occur. Tumor growth was local: there was no evidence of macroscopic metastases or invasion to the surrounding host tissues.

Measurement of tumor size

After inoculation, the tumor was measured twice weekly (length, breadth and depth) using a slide caliper. Experiments were concluded 4 weeks after the day on which treatment was begun.

Histologic and histochemical studies

The tumors were excised and fixed in formalin, and sections were stained with hematoxylin and eosin. Some specimens were left overnight in a refrigerator in formal calcium and cut in the cryostat for demonstration by the method of Burstone, using naphthol AS-BI phosphate with fast red violet LB salt as the coupler for alkaline phosphatase [13, 14].

Statistical analysis

After a 4-week observation period an analysis was made of the growth curves [15]. Tumor growth in each group was compared with the corresponding controls and differences were assessed by Student's t test.

EXPERIMENTS AND RESULTS

Tumor growth curves

In the first experiment, 24 hr after inoculation of tumor blocks HuIFN-α was administered i.p. 3 times a week in doses of 25,000 and 50,000 IU/mouse to the animals of the two respective groups. Significant inhibitory effects were observed in the group treated with 50,000 IU of HuIFN-α compared with the control group ($P < 0.001$). Figure 2 shows the results with HuIFN-α given in doses of 25,000 and 50,000 IU/mouse. In the second experiment, 14 days after inoculation of tumor blocks, when the tumor had reached a moderate size (average diameter, 4 mm), HuIFN-α (50,000 IU/mouse) was administered i.p. 3 times weekly. Differences in tumor size between the control group and the HuIFN-α-treated group were significant at $P < 0.01$ (Fig. 3). In the third experiment, 14 days after inoculation of tumor blocks, when tumor length and breadth were about 6 mm, HuIFN-α was administered daily in a dose of 50,000 IU/mouse (Fig. 4). A significant inhibitory effect was observed in the treated group compared with the controls ($P < 0.001$).

Histologic studies

In the OS-OH treated with 25,000 IU of HuIFN-α there were no changes in the tumor cells (Fig. 5). In those treated daily with 50,000 IU of HuIFN-α, however, the changes were marked fibrosis and pycnosis of tumor cells and there was evidence of mitosis (Fig. 6).

Histochemical studies

In the control group tumor cells showed considerable alkaline phosphatase activity (Fig. 7A). In the group intermittently treated with 50,000 IU/mouse, however, there was some evidence of alkaline phosphatase activity (Fig. 7B). In the group treated daily with HuIFN-α the tumor cells showed no alkaline phosphatase activity.

DISCUSSION

In 1970 Gresser *et al.* observed an increased survival in BALB/c mice inoculated with 10^3–10^4 EA cells and treated i.p. with mouse-brain IFN (20,000 IU), thereby suggesting that the mouse-brain IFN inhibited the multiplication of the tumors [3]. In 1977 the L cell growth-inhibiting capacity of poly(I)·poly(C) in nude mice was directly compared with that of IFN by De Clercq [16]. At 10^5 IU/mouse it caused some inhibition comparable to that observed with 1 μg of poly(I)·poly(C). Strander reported that 27 patients with osteosarcoma received treatment with HuIFN-α and that 60% of these patients were disease-free at 2 yr, whereas 10% of the historical control patients had no evidence of the disease at 2 yr [17]. He had used IFN as adjuvant therapy for all the patients but observed no reduction or disappearance of the macroscopic lesion. In our experiment OS-OH was markedly sensitive to IFN and there was not only an inhibition of the tumor growth but also a reduction or disappearance of the tumor.

Concerning the direct oncolytic action of IFN, reduction or disappearance of the tumor was observed with the administration of IFN 2 weeks after tumor inoculation, a time when the tumor

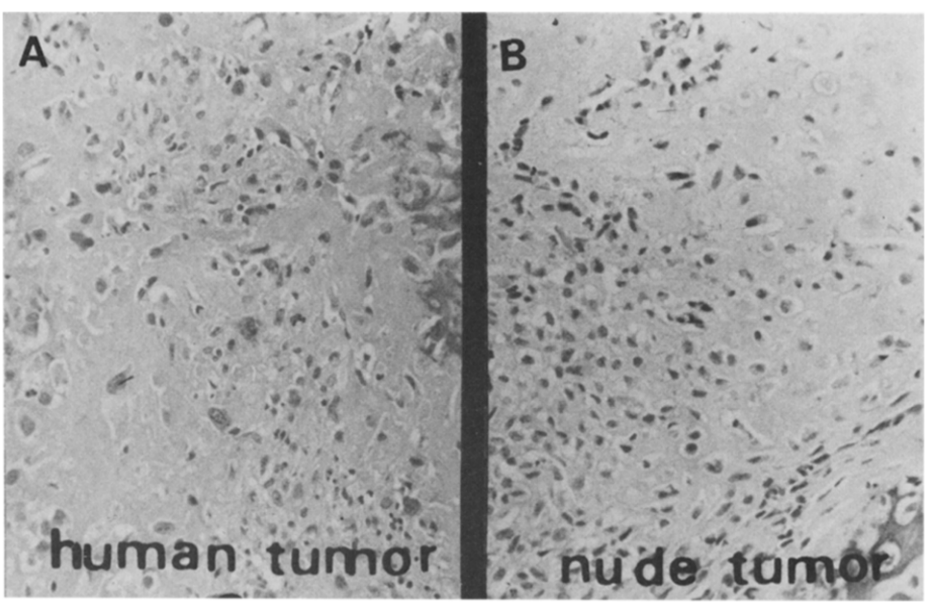

Fig. 1. Histology of OS-OH, derived from a pulmonary metastasis of an osteosarcoma of the right femur of a 21-yr-old male student. (A) Tumor used for transplantation; (B) 9th-passage tumor in nude mice.

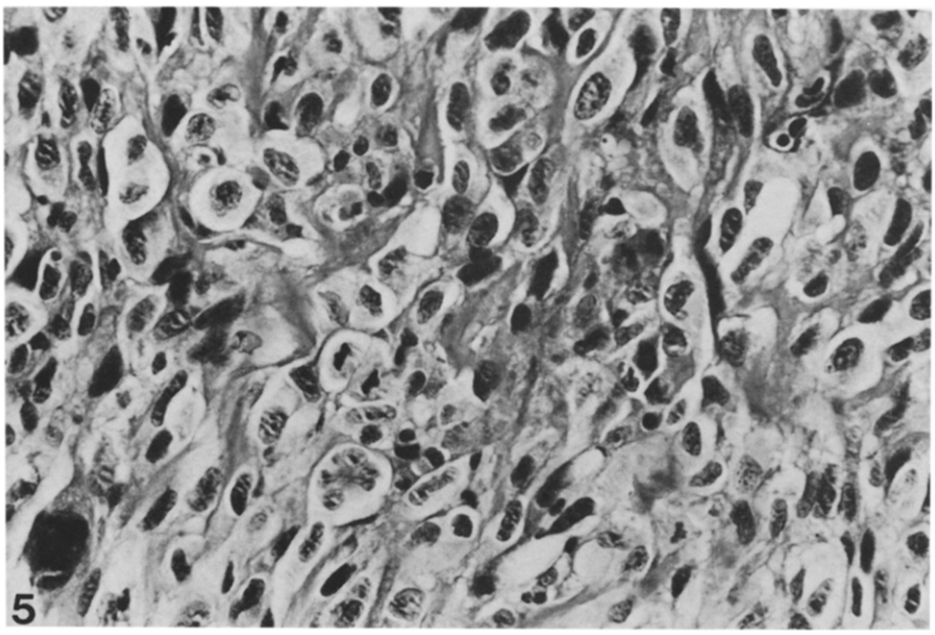

Fig. 5. Histology of OS-OH (passage 4) treated with 25,000 IU of HuIFN-α (administered 3 times a week). ×200.

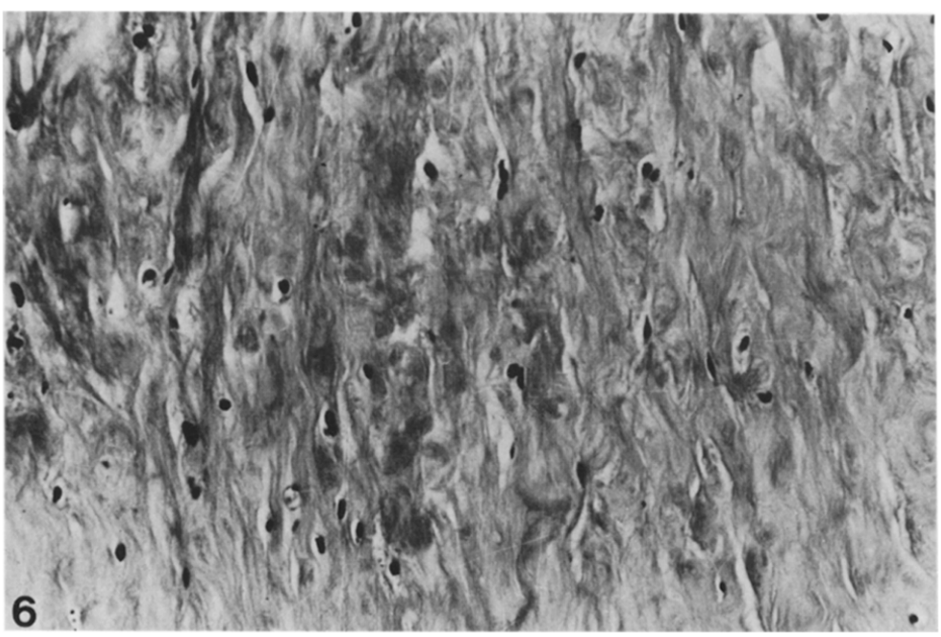

Fig. 6. Histology of OS-OH (passage 9) daily treated with 50,000 IU of HuIFN-α. ×200.

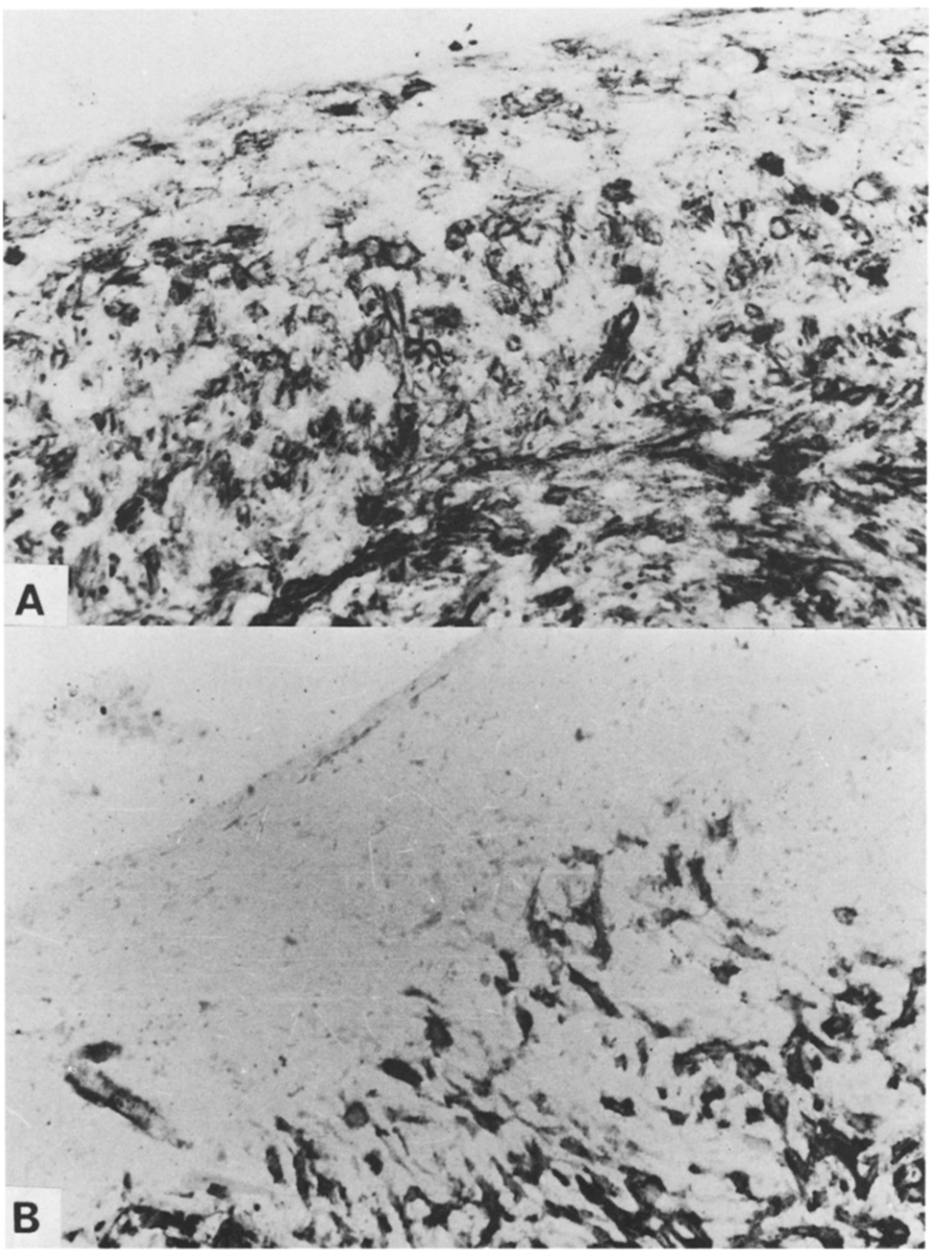

Fig. 7. Histochemistry for alkaline phosphatase OS-OH (passage 9). (A) Alkaline phosphatase activity in the untreated group; (B) some phosphatase activity in the group intermittently treated with HuIFN-α.

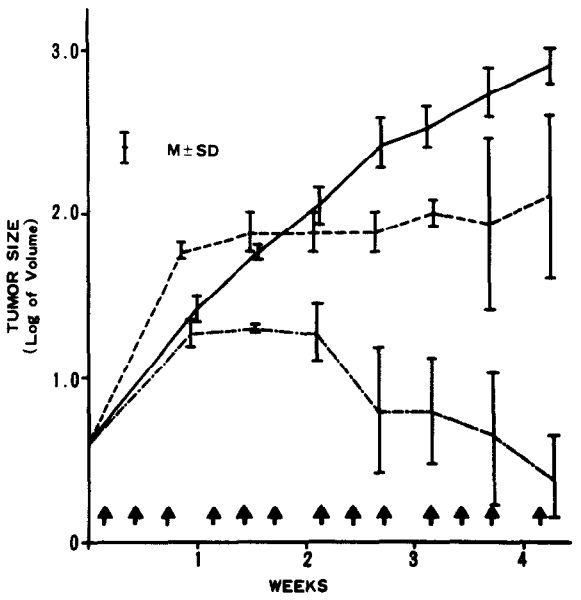

Fig. 2. Growth curves of OS-OH (passage 4) in nude mice treated with HuIFN-α (either 25,000 or 50,000 IU/mouse). ----- HuIFN-α 25,000 IU/mouse; -·-·- HuIFN-α 50,000 IU/mouse; ——— control (P < 0.001).

had reached a moderate size (4–6 mm). These results show that IFN may have a direct oncolytic action which directly inhibits tumor growth.

In the non-treated group tumor cells showed considerable alkaline phosphatase activity, principally in the cell membranes. In striking contrast, HuIFN-α-alone-treated tumor cells were all but devoid of alkaline phosphatase activity. As this enzyme is considered to be a key one in the formation of the mesenchymal matrix, that is, an indicator of the function of osteoblasts, absence of the enzyme indicates a pronounced cytotoxic effect of the tumor cells [14]. In clinical applications, although it is difficult to calculate the units of IFN used for nude mice in terms of the dosage for humans, at least $1–2 \times 10^7$ units of IFN may be required in a single dose to treat osteosarcoma in humans. Ban *et al.* reported that HuIFN-α had a transient effect in two of four patients with osteosarcoma with a pulmonary metastasis after the amputation of an affected limb [18]. The dose of HuIFN-α given was $3–5 \times 10^6$ IU twice a week. We conclude from this study that IFN may prove to be a most effective agent for treating osteosarcoma.

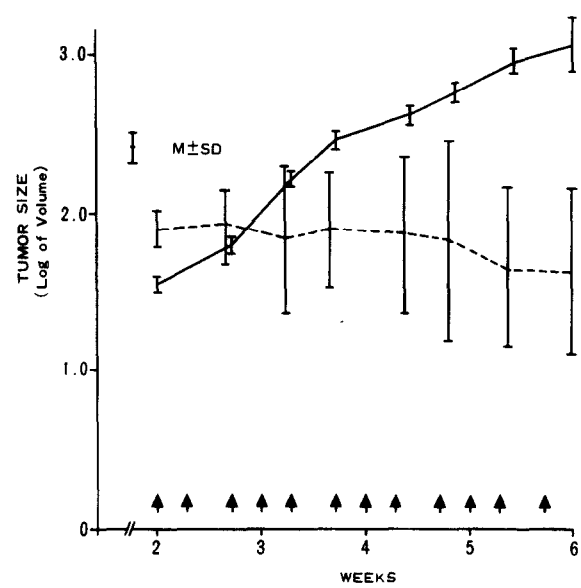

Fig. 3. Growth curves of OS-OH (passage 6) in nude mice. HuIFN-α treatment was initiated 14 days after tumor inoculation. ----- HuIFN-α 50,000 IU/mouse; ——— control (P < 0.01).

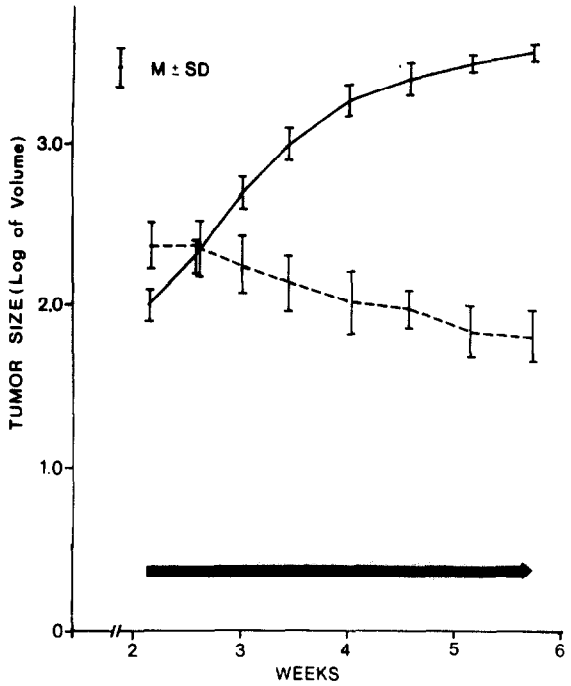

Fig. 4. Growth curves of OS-OH (passage 9) in nude mice. 50,000 IU of HuIFN-α was administered i.p. daily for 26 days (thick line). ------- Treated group; ——— control (P < 0.001).

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