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SHORT COMMUNICATION

EFFECT OF HUMAN LYMPHOBLASTOID INTERFERON ON HUMAN YOLK SAC TUMORS IN NUDE MICE[1]

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The effect of human lymphoblastoid interferon on the growth of human tumors heterotransplanted into nude mice was examined. The human tumor lines examined, named YST-1, YST-2 and YST-3, were derived from yolk sac tumors of the ovary. Daily intraperitoneal injection of 3×10^4 U interferon per mouse for 14 days did not inhibit the growth of any of these three human tumor lines. A close correlation was observed between the tumor volume and the level of alpha-fetoprotein in sera of mice bearing the YST-1 tumor ($r=0.55$) or YST-2 tumor ($r=0.70$). No histological differences were detected between tumor cells of interferon-treated and control mice. Tumor-bearing mice treated with interferon showed no marked weight loss.

Interferon has been recognized to have potential clinical value for inhibiting growth of malignant tumors (Billiau, 1981; Gresser et al., 1978; Strander, 1977; 1978), but the mechanism of its antitumor effect is not yet fully understood. Clinical trials on the antitumor effect of interferon are limited by the small amounts of interferons available, and so laboratory studies using animal models of human cancer seem desirable. Since Rygaard and Povlsen (1969) first reported the heterotransplantation of human tumors into nude mice, nude mice have been widely used in cancer research. However, although the human-nude mouse system seems to have the advantage that the direct action of interferon on human tumors can be examined with a small mount of interferon, there have been only a few reports so far on the potential of interferon as an antitumor agent in this system (Balkwill et al., 1982; Balkwill et al., 1980; De Clercq, 1982; De Clercq et al., 1978; Horoszewicz et al., 1978; Yokota et al., 1976).

We have established three human yolk sac tumors of the ovary (YST-1, YST-2 and YST-3 lines) in BALB/C female nude mice (nu/nu), which were obtained from Clea Japan, Takatsuki, Osaka. Using these tumors we examined the effect of human lymphoblastoid interferon on the growth of human tumors.

1 A part of this work was presented at the 13th International Cancer Congress in Seattle in September, 1982.

TABLE 1. *Summary of results*

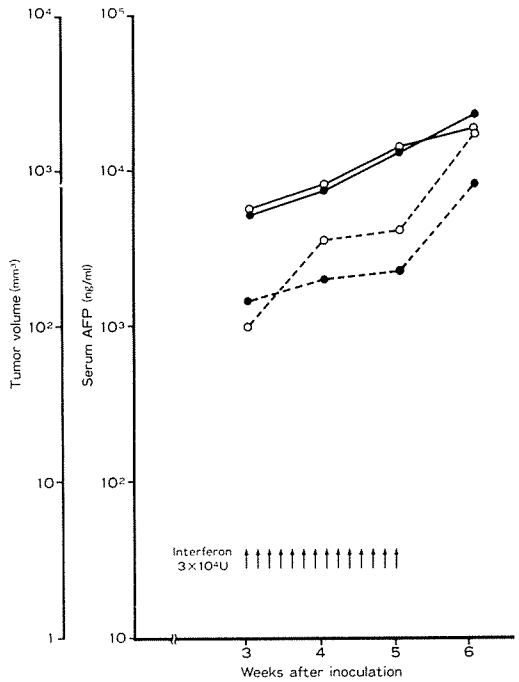
Tumor line / Treatment	YST-1 NaCl	YST-1 Interferon	YST-2 NaCl	YST-2 Interferon	YST-3 NaCl	YST-3 Interferon
Number of mice	5	5	5	5	5	5
Tumor weight (g) (mean±SD)	1.77±0.32	1.63±0.66	2.54±0.60	1.67±0.72	1.25±0.94	4.02±0.82
T/C%[a] by weight	92.4		66.0		321.1	
T/C%[a,b] by volume	84.7		79.2		142.0	

[a] T/C% = mean for treated mice/mean for control mice × 100.

[b] The values shown were calculated from final measurements of tumor volume.

The rate of take of all the tumor lines was almost 100%. Moreover all three tumor lines could produce alpha-fetoprotein (AFP). Detailed characteristics of these heterotransplantable tumors were reported previously (Sawada et al., 1981; Sawada et al., 1982). Human lymphoblastoid interferon (specific activity 10^7 U/mg protein) supplied by Hayashibara Biochemical Laboratories Inc. (Okayama, Japan) was used for experiments. The properties of this interferon were reported previously by Imanishi et al. (1980). A dose of 3×10^4 U of interferon in 0.2 ml of 0.9% NaCl solution was injected intraperitoneally into the mice for 14 days; control mice received the same volume of 0.9% NaCl. The effect of interferon was evaluated by calculating T/C%, the tumor volume or weight in treated mice as a percentage of that in control mice, as we described in a recent report (Sawada et al., 1983). At the end of experiments, all the treated and control tumors were resected and weighed, and T/C% by weight was calculated. For examining the correlation between the serum AFP level and tumor volume, blood samples (30 μl) were taken at regular intervals from a tail vein of each mouse, and AFP was measured with an Eiken AFP radioimmunoassay kit (Eiken Immunochemical Laboratory, Tokyo) by Mr. K. Iwanaga in Osaka Kessei Research Laboratories. AFP values in the sera of nude mice without tumors were less than 5 ng/ml, the lowest limit for detection of AFP with this kit. At the start of treatment, the AFP levels of tumor-bearing mice ranged from 714 to 1232 ng/ml for mice with YST-1, 180 to 833 ng/ml for those with YST-2 and 5 to 150 ng/ml for those with YST-3. To monitor the toxicity of the drugs, we weighed the mice once a week during the experiments. Student's t test was used for statistical analysis.

YST-1 tumor at the 17th passage was transplanted into 2 groups of 5 mice each, which were then treated daily with 3×10^4 U of interferon and 0.9% NaCl respectively. Tumor growth was only slightly suppressed by inter-



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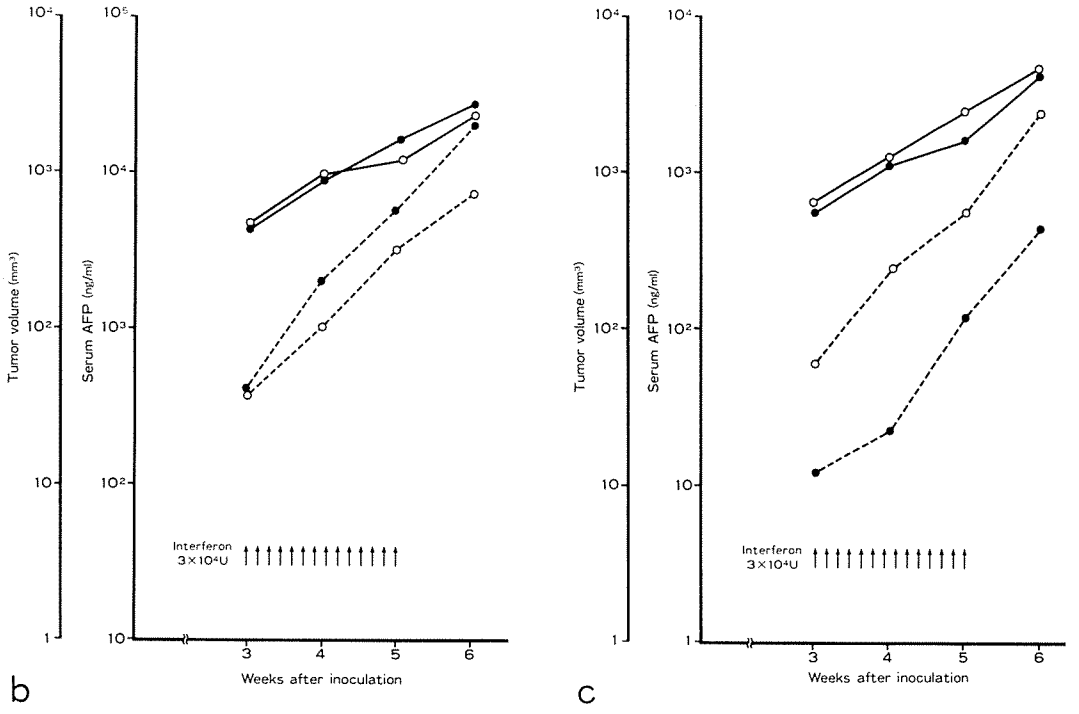

FIGURE 1. Changes in tumor volume (—) and the level of serum AFP (···) during treatment with 0.9% NaCl (●) or interferon (○). a, YST-1 tumor; b, YST-2 tumor; c, YST-3 tumor.

feron as shown in Fig. 1a: the mean weight of tumors in the interferon-treated group was 1.63 ± 0.66 g, which was not significantly different from that of tumors in the control group (1.77 ± 0.32 g). The T/C% by weight was 92.4 and the maximal T/C% by volume was 84.7 (Table 1).

YST-2 tumor at the 9th passage was transplanted into 10 mice, which were treated as these with YST-1 tumor. Tumor growth was only slightly suppressed by interferon treatment (Fig. 1b): there was a small difference in the tumor weights of control (2.54 ± 0.60 g) and interferon-treated (1.67 ± 0.72 g) mice, but this was not statistically significant. The T/C% by weight was 66.0 and the maximal T/C% by volume was 79.2 (Table 1).

YST-3 tumor at the 7th passage was transplanted into 10 mice which were also treated daily with 3×10^4 U of interferon or 0.9% NaCl for 14 days. For some unknown reason the growth of tumors was increased by interferon (Fig. 1c). The mean weight of tumors in the interferon-treated group was 4.02 ± 0.82 g, which was more than that of tumors in the control group (1.25 ± 0.49 g). The T/C% by weight was 321.1 and the maximal T/C% by volume was 142.0 (Table 1).

There was little difference in the body weights of mice in interferon-treated and control groups and no marked weight loss of tumor-bearing mice was recognized after treatment with interferon (Table 2).

There are several reports that human interferon inhibits the growth of human tumors heterotransplanted into nude mice. In these previous studies, human leukocyte, lymphoblastoid or fibroblast interferon was injected intraperitoneally and human tumors were inoculated subcutaneously as in this study. Yo-

TABLE 2. *Body weights of tumor-bearing mice before and after treatment*

Tumor line	YST-1		YST-2		YST-3	
Treatment	NaCl	Interferon	NaCl	Interferon	NaCl	Interferon
Number of mice	5	5	5	5	5	5
Weight of mice (g) (mean±SD)						
Before treatment	25.7±1.5	25.0±2.2	23.5±1.1	23.1±0.9	24.8±3.0	22.9±1.3
After treatment	26.2±1.8	26.8±1.5	25.9±1.2	24.6±1.1	27.7±2.3	28.4±0.6

kota et al. (1976), Horoszewicz et al. (1978) and Balkwill et al. (1980) reported that $2-2.5\times10^4$ U of interferon inhibited tumor growth. On the other hand, De Clercq et al. (1978), Horoszewicz et al. (1978) and Balkwill et al. (1982) reported that 2×10^4 U of interferon did not affect the growth of xenotransplanted tumors. In the present work we also found that 3×10^4 U of human lymphoblastoid interferon did not inhibit the growth of 3 yolk sac tumors. These results suggest that tumors differ in sensitivity to interferon. We suppose human yolk sac tumors, which are highly malignant, are not sensitive to human lymphoblastoid interferon by nature.

No characteristic cytological nor histological differences were seen between the tumor cells in interferon-treated and control mice. Moreover, histologic examination showed no evidence of increased lymphocyte or macrophage infiltration into tumor tissue after interferon treatment. As suggested by others (De Clercq, 1982), the antitumor activity of interferon may be due to its direct action on the tumor cells, on the host surveillor cells, or on both. Our results suggest the absence of a direct action of interferon on yolk sac tumor cells.

A close correlation was found between the tumor volume and the level of AFP in sera of mice bearing YST-1 ($r=0.55$) or YST-2 ($r=0.70$), but not YST-3 tumors. Although, AFP is considered to be the most useful marker of yolk sac tumors, Talerman et al. (1982) pointed out that the presence of an elevated level of a certain tumor marker indicates only that the tumor elements responsible for its production are present. The intratumor element that produces AFP has not yet been identified.

This preliminary experiment failed to demonstrate an antitumor potential of interferon on heterotransplantable tumors. Studies on the effects of various types of interferons on tumor are now in progress.

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