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PRELIMINARY REPORT

ANTITUMOR EFFECTS OF INTERFERON ON TRANSPLANTED TUMORS IN CONGENITALLY ATHYMIC NUDE MICE

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Gresser et al. (1969 a, b; 1970) first reported the antitumor effects of interferon on transplantable mouse tumors. Since then there have been many reports on the effects of interferon, not only on transplantable animal tumors, but also on virus induced or spontaneous tumors. However, it is still uncertain whether the antitumor effects of interferon are direct or whether they involve the defence mechanism of the host animal. Congenitally athymic nude mice (Flanagan, 1966; Pantelouris, 1968) are known to be practically devoid of T-cell function. Thus, heterologous human tumor cells can be transplanted into nude mice and the effects of interferons of different origins on their growth can be examined. This paper reports the effects of mouse and human interferons on mouse and human tumor cells transplanted into nude mice. For comparison, the effects of these interferons on transplanted mouse tumor cells in ordinary mice were also investigated.

Female nude mice (BALB/c-*nu*/*nu*) were purchased through Dr. Normura of the Central Institute for Experimental Animals, Kanagawa. The mice were bred in flexible film isolators under specified pathogen-free conditions, mating BALB/c-*nu*/+ females with BALB/c-*nu*/*nu* males. They were proved to be free from most pathogenic microorganisms of laboratory mice including mouse hepatitis virus and hemagglutinating virus of Japan (HVJ). Female mice of 5 weeks of age were used in all experiments. They were housed 3 mice to a cage in plastic cages covered with filter cap to prevent microbial contamination and the cages were kept in a cubicle at 22–25 C. The mice were fed on mouse food for germ-free mice CL-2 (CLEA Japan Inc., Tokyo) and tap water sterilized at 120 C for 20 min. Mouse interferon was obtained from the brains of mice infected with Japanese encephalitis virus, strain JaGAr as follows. A 20% emulsion of infected mouse brain in phos-

phate buffered saline, pH 2.0 was stood for 2 days at 4 C. Then it was centrifuged at 10,000 rpm for 60 min at 4 C and the supernatant was adjusted to 80% saturation of ammonium sulfate. The resultant precipitate, containing most of the interferon activity, was further purified by adsorption on CM-Sephadex C-50 and by precipitation with ammonium sulfate. Details of the purification procedure will be published elsewhere. Interferon was assayed as reported before (Yokota et al., 1975) with vesicular stomatitis virus strain New Jersey on cultures of L cells by measuring reduction in yield. The purified preparation of mouse interferon contained 36,000 IU/mg protein. For comparison, human interferon was prepared from human leukocytes by the procedure of Matsuo et al. (1974). Briefly this procedure was as follows. Human leukocytes were separated and purified from buffy coats and suspended in Eagle's minimum essential medium supplemented with 5% inactivated human serum. Then they were infected with UV-irradiated Newcastle disease virus, strain Miyadera and cultured for 22 hr at 37 C with shaking. The culture was then centrifuged and the supernatant was adjusted to 70% saturation of ammonium sulfate. The precipitate was further purified by chromatographies on CM-cellulose and DEAE cellulose columns and reprecipitation with 70% saturation of ammonium sulfate. The purity of the human interferon used in this experiment was 80,000 IU/mg protein. For testing the antitumor effects of the interferons, nude mice were injected subcutaneously with 5×10^5 HeLa cells in Eagle's minimum essential medium supplemented with 5.0% calf serum. Doses of 25,000 IU of human or mouse interferon were injected intraperitoneally into each mouse twice a week for 3 weeks starting when the tumor nodulus became palpable. Control mice were given physiological saline instead of interferon. Antitumor effects were estimated from the mean diameter of tumors in mm measured at intervals during the experiment. Each tumor was excised and weighed on the 50th day after the first injection of interferon. For comparison, the effects of interferons were tested on subcutaneously transplanted ascitic Sarcoma 180 cells both in nude and ICR-JCL mice (purchased by CLEA Japan Inc., Tokyo). For this, each mouse was injected subcutaneously with 1×10^6 cells of ascitic Sarcoma 180 which had been maintained by serial passage in ICR-JCL mice. Interferon was administered in the same way as for mice with HeLa cells except that the first injection was given 1 week after tumor transplantation and tumors were excised on the 30th day after administration of interferon.

As seen in Table 1, the growth of Sarcoma 180 was significantly inhibited both in nude and ordinary mice by mouse interferon but not by human interferon. Thus even in nude mice the growth of transplantable mouse tumor is inhibited by interferon and species

TABLE 1. *Antitumor effects of interferon on subcutaneously transplanted Sarcoma 180 in BALB/c-nu/nu and ICR-JCL mice*

Interferon	Tumor weight (g)[a]	
	BALB/c-*nu/nu*	ICR-JCL
Control (saline)	13.2±1.3[b]	9.1±2.5
Mouse	4.2±0.7 (68.2%)[c]	5.3±1.2 (41.8%)
Human	11.0±2.4 (1.4%)	8.6±1.1 (5.4%)

[a] Measured 30 days after the first administration of interferon.
[b] Average of 6 mice with SE.
[c] Per cent inhibition.

TABLE 2. *Antitumor effect of interferon on subcutaneously transplanted HeLa cell in BALB/c-nu/nu mice*[a]

Interferon	Tumor size (mm)	Tumor weight (g)
Control (saline)	19.2±2.3[b]	1.8±0.5
Mouse	19.0±3.8	2.1±1.2
Human	13.5±1.6	0.8±0.3

[a] Measured 50 days after the first administration of interferon.

[b] Average of 3 mice with SE.

specificity of its action was demonstrated in nude mice. Table 2 shows results on HeLa cells. Growth of transplanted HeLa cells in nude mice was clearly inhibited by human interferon, eventhough only a few mice were used in the experiment. Kishida et al. (1973) reported in vitro activation of macrophages by interferon and they suggested that this activation played some significant role in the antitumor effect of interferon in vivo. Our results on nude mice do not exclude this possibility but they provide evidence for a direct effect of interferon on tumor cells. Further studies are in progress on the mechanism of the antitumor effect of interferon especially in nude mice.

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