

Short Communication

Failure of high doses of α interferon to affect the growth of human carcinoma, melanoma, and myeloid leukaemia xenografts

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Balkwill *et al.* (1980) observed that human leukocyte interferon (IFN) from lymphoblastoid cells prevented 2/3 human mammary carcinomas from growing as xenografts in genetically athymic nu/nu mice, but failed to slow the growth once the tumours had been established. Subsequently, Balkwill *et al.* (1982) found a mammary tumour, the growth of which as a xenograft was stopped by IFN- α when given 2 or 3 weeks after implantation. We failed to observe any effect of a highly purified

human IFN- α on a human malignant melanoma xenograft (HX34; Selby *et al.*, 1980), a human lung adenocarcinoma xenograft (HX70; Shorthouse *et al.*, 1980) and a xenograft of acute myeloid leukaemia (AML) cells in immunodeprived mice (Table I). The present experiments differed from those carried out by Balkwill *et al.* (1980) not only in the type of tumour employed, but also in the source of IFN- α and the nature of the murine host.

We used IFN- α 2 produced in *E. coli* by direct

Table I Effect of interferon on s.c. human tumour xenografts

Recipient: Thymectomised—total-body irradiated CBA mice: these mice are leukopenic for the first 7 days but a cross-species effect of IFN on host leukocytes would neither be detectable nor expected in this system. (Grafting procedures as described in Palu *et al.*, 1979; Selby *et al.*, 1980; & Shorthouse *et al.*, 1980).

Interferon: 10^7 u kg⁻¹ per day i.p. starting one day before tumour implant for 14 days. No evidence of toxicity at this dose.

<i>Tumour</i>			<i>Incidence of tumours</i>	<i>Average volume (mm³) at day 18 (\pms.e.)</i>
Adenocarcinoma of lung (HX70): s.c. transplant from established xenograft line	Exp. 1	Control	8/8	295 \pm 44 ^a 178 \pm 29 ^a <i>P</i> < 0.05
		IFN	10/10	
	Exp. 2	Control	10/10	153 \pm 33 186 \pm 24
		IFN	10/10	
Melanoma (HX34): s.c. transplant from established xenograft line		Control	10/10	442 \pm 114 473 \pm 179
		IFN	7/8	
AML cells (2×10^7 s.c.) taken from peripheral blood and stored in liquid N ₂		Control	17/20	98 \pm 10 106 \pm 8
		IFN	19/20	

^aGrowth rate from Day 18 to Day 25 is the same in both groups.

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expression of cloned DNA while Balkwill *et al.* (1982) used IFN isolated from tissue culture supernatants. Both preparations were of comparable purity with a biological activity in the range of 10^8 u mg^{-1} (Wetzel *et al.*, 1981). While not chemically identical, the biological properties of different preparations of IFN- α are extremely similar (De Grado *et al.*, 1982) and there is little reason to attribute our inability to demonstrate an effect of IFN on human tumour xenografts to a difference in the IFN used. The dose administered by us (10^7 u kg^{-1} per day i.p.) was 10 times greater than the $2 \times 10^4 \text{ u}$ per mouse per day s.c. used by Balkwill *et al.* (1980) to prevent take and equal to the dose (i.e. $2 \times 10^5 \text{ u}$ per mouse per day s.c.) needed to stop the growth of the established mammary carcinoma. The other difference is in the nature of the immunosuppressed mice used to grow the xenografts. Balkwill *et al.* (1980, 1982) employed nu/nu mice whereas in our experiments the human tumours were grown in CBA mice deprived of T-cells by thymectomy at 4 weeks of age and 9 Gy total body irradiation at 8 weeks, the lethal effects of which were prevented by pre-treatment with 200 mg kg^{-1} Ara-C (Millar *et al.*, 1978).

At the time when the human tumours were implanted in these irradiated mice the animals were leukopenic. Again, however, this difference is unlikely to account for the failure to demonstrate an effect of human IFN- α on the tumour xenografts used by us since Balkwill *et al.* (1982) have provided convincing evidence that the effect observed by them is a direct one of the IFN on the mammary tumour cells. The species specificity of the action of IFN is such that no effect on *host* resistance mechanisms (e.g. NK) would have been expected. In their experiments the human IFN- α did not stimulate the activity of murine NK cells, nor did it induce 2-5 adenylic acid synthetase in mouse tissues, but it raised this enzyme in the human carcinoma xenograft following administration of IFN to nu/nu mice.

The dose of highly purified human IFN- α used in these experiments, 10^7 u kg^{-1} per day, caused no detectable toxicity in the mice and it would have been possible to give substantially greater amounts. However, on a weight basis the protocol employed

is ~ 3 times the dose found by Rohatiner *et al.* (1982) to be maximally tolerated in man, and over 50 times the human dose used by Priestman (1980).

In the first experiment (Table I) IFN caused a slight slowing of the growth of the adenocarcinoma of lung, which was significant at the $P < 0.05$ level. However, in a second experiment no such effect was noted. The melanoma grows in immunodeprived mice both s.c. and when given i.v. in the lung (Table II); IFN had no effect on the growth at either site. In the majority of the cell populations that have been studied (Alexander, 1982) acute myeloid leukaemia (AML) cells, taken from the blood of patients with a blood cell separator, grow in CBA mice that have been immunosuppressed by thymectomy and total body irradiation. However, almost invariably after 2-3 weeks the local tumours which are made up of $> 90\%$ of human cells regress spontaneously (Palu *et al.*, 1979).

Table II Effect of interferon on the lethality of human melanoma xenografts grown as lung tumours
 4×10^6 melanoma cells injected i.v. (HX34)

	Dead by 90 days	Median day of death
Control	4/5	44 days
IFN	5/5	42 days

(all deaths due to multiple lung tumours).

Surface marker and histochemical studies (Palu *et al.*, 1979; Forbes *et al.*, 1981) indicate that the regression is associated with maturation of the AML cells; however, this process is not hastened by administration of IFN- α under the conditions of the experiment described in Table I.

No other tumour xenografts, including the lung adenocarcinoma (Shorthouse *et al.*, 1980) and melanoma (Selby *et al.*, 1980) treated with IFN, regressed spontaneously in these immunodeprived mice.

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