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Human Lymphoblastoid Interferon Can Inhibit the Growth of Human Breast Cancer Xenografts in Athymic (Nude) Mice

F. BALKWILL,* J. TAYLOR-PAPADIMITRIOU,* K. H. FANTES† and A. SEBESTENY*

**Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London, WC2A 3PX, United Kingdom; and*
†*Wellcome Research Laboratories, Langley Court, Beckenham, Kent, United Kingdom*

Abstract—*Human lymphoblastoid (Namalwa) interferon inhibited the development and growth of 2 out of 3 human breast carcinomas grown as xenografts in athymic (nude) mice. This inhibition could be abolished by preincubation of the interferon with a potent antiserum.*

INTRODUCTION

THE INTERFERONS are a group of glycoproteins that exert a potent anti-viral effect [1, 2] but they can also inhibit the growth of a wide range of normal and tumour derived cells *in vitro* [3, 4] and have important immunoregulatory functions [5]. Furthermore, in animal tumour model systems it is now well documented that interferons are capable of delaying neoplastic progression whether in spontaneous, transplantable, chemically or virally induced cancers, the degree of suppression often being comparable to that of conventional chemotherapeutic agents (for reviews see refs [2] and [4]). Clinical trials to test the anti-tumour effect of interferons in man have been limited by the availability of human interferons prepared from blood bank leucocyte or fibroblast sources [6]. However, the judicial use of leucocyte interferon in a small number of patients with non-Hodgkin's disease lymphoma [7] and myelomatosis [8], where disease can be easily monitored, has shown an antitumour effect. Similarly, this interferon, administered after surgery to patients with osteosarcoma, suppressed or delayed the development of metastases [9]. To obtain statistically significant data on the effects of interferons in human cancer would require treatment of large numbers of patients for long periods of time, using greater amounts of interferons than are currently being produced. A preliminary approach to this problem could be the treatment of human tumours grown as xenografts in athymic (nude) mice [10]. In the last 2 years we have established 3 primary human breast carcinomas as transplantable xenografts in nude mice [11]. These tumours are slow growing, retain their original morphology and surface phenotype, and like the cells from the original carcinoma, the cells from the xenografts do not grow in the culture systems available [11]. We now find that the development and growth of these tumours can be inhibited by a human interferon prepared from human lymphoblastoid (Namalwa) cells, [12] which will soon be available for clinical trials in man (N. Finter personal communication).

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MATERIALS AND METHODS

Athymic (nu/nu) mice

The establishment of the random bred nu/nu colony of mixed genetic background has been described previously [11]. All experiments used female nu/nu mice, aged between 4 and 10 weeks, which were housed in a negative pressure isolator (Vickers Medical, Basingstoke, U.K.), with high efficiency air filtration. All routine isolator supplies were sterilized either by heat or 2.5 Mrad gamma irradiation, and all items entering or leaving the isolator were sprayed with 3% Hycolin in the entry lock. The life span of mice kept under these conditions was comparable of normal SPF mice and no outbreaks of infectious disease were encountered.

Transplantation of the tumours

Selected pieces of tumours growing in nu/nu mice were cut into cubes of 1–8 mm^3 and 4 such fragments were loaded into Bashford needles and implanted into 4 different ventral sites per mouse. Further pieces of the tumour were retained for histology and immunofluorescence at each transplant. The mice were inspected once weekly and all tumours over 2 mm^3 in size were recorded.

Immunofluorescent staining of tumours

Cell suspensions were prepared from excised tumours and immunofluorescence was carried out as described [11] with an anti-mouse serum and an antiserum prepared against the milk fat globule and specific for human mammary epithelial cells (anti-HME) [11, 13]. The tumours were examined at every passage, and in all three the majority of cells were positive with the anti-HME sera, although a constant fraction (10–20%) stained with the anti-mouse serum.

Interferon assay

Human interferon levels were measured in a plaque reduction assay using V_3 cells [14] and Semliki Forest virus and calibrated using British Reference Standard 69/19. (National Institute of Biological Standards and Controls, Hampstead, London, U.K.). An internal standard was employed in every assay.

Interferon

Two batches of human Namalwa interferon, purified by a combination of TCA precipitation, ethanol extraction, and affinity chromatography, were used in the experiments. Batch code 471/6 had a spec. act. of 4.2×10^6U/mg protein and Batch code 580/22, a spec. act. of 5.6×10^7U/mg protein. All interferon was diluted where necessary in phosphate-buffered saline (PBS) or PBS + 1% foetal calf serum. (FCS) (Gibco Biocult Ltd., Paisley, U.K.).

The anti-interferon globulin was prepared by repeated inoculation of 3 New Zealand white rabbits with increasing doses of 5×10^5–8×10^6U of semi-purified lymphoblastoid interferon. Bleeds from several dates were pooled, the globulins prepared by ammonium sulphate precipitation, dialysed and further concentrated by pressure filtration using an Amicon P30 membrane. One millilitre of the final concentrate neutralised greater than 10^6U interferon.

Interferon or control injections were given subcutaneously in the skin over the peritoneum in volumes of 50 μl.

Tumours

All implanted tumours were primary infiltrating carcinomas of the breast (Malignancy Grade III) from postmenopausal women.

Tumour 630. The first transplant was carried out at 12 weeks after implantation and the tumour has now been through 5 passages. The average time between transplants was 15 ± 4 weeks.

Tumour 534. The first transplant of 534 was carried out 33 weeks after implantation and the tumour has now been through 6 transplants. The average transplant time was 13 ± 2 weeks.

Tumour 236. The first transplant was carried out 39 weeks after implantation and the tumour has now been serially transplanted for 6 passages to date. Average transplant time was 19 ± 11 weeks.

RESULTS

Preliminary experiments indicated that significant levels of human interferon could be found in the sera of nude mice after subcutaneous injection: in a typical experiment, an initial injection of 1×10^4U of interferon 580/22 gave serum levels of 188U, 383U and 339U/ml at 30 min, 2 hr and 6 hr post injection respectively. By 24 hr there was no measurable interferon in the serum. The human interferon was not antigenic in these mice: mice that had been treated with 2×10^4U interferon daily for 12 weeks showed no specific neutralising antibodies to interferon when compared to control mice (data not shown).

Tumour 630

In the first experiment, this tumour, at passage 2, was transplanted into 19 mice which were given either 1×10^4U of interferon 471/6 or a control injection of PBS 3 times weekly. After 13 weeks 9/9 control mice and 8/10 interferon treated mice had developed tumours and all mice were killed and the tumours excised and weighed. A small difference in mean tumour weight per mouse between control mice (3.73 g) and interferon treated mice (2.4 g) was noted but this was not statistically significant. A further experi-

Table 1. *Effect of interferon treatment on number and weight of tumours developing as xenografts in nude mice*

Experiment	A(630)		B(534)		C(236)	
Animals injected with:	Placebo	Interferon	Placebo	Interferon	Placebo	Interferon
Number of mice with tumours/total number of mice	6/9	3/9	9/9	5/9	7/7	8/8
Mean weight tumours per tumour-bearing mouse (g ± S.D.)	1.24 ± 0.76	0.45 ± 0.16	1.22 ± 1.48	0.58 ± 0.38	2.44 ± 1.31	2.42 ± 2.78
Total number of tumours	19	3	29	11	38	32
Mean weight per tumour (g)	0.41	0.45	0.38	0.26	0.45	0.61

For experimental details see text and legend to Fig. 1.

ment was therefore carried out in which level and frequency of dose were increased. Tumour 630 at passage 4 was transplanted into 2 groups of 9 mice which were then given either a control injection of PBS + 1% foetal calf serum (FCS), or 2×10^4U of interferon 580/22 daily for 13 weeks. As shown in Fig. 1A and Table 1A, this dosage of interferon had a dramatic effect on the number of tumours produced (3 in the interferon group, 19 in the control group), the mean weight of tumour per tumour-bearing mouse (0.45 g in the interferon group, 1.24 g in control mice), but not on the mean weight of individual tumours. Also, as shown in Fig. 1A, the interferon treatment delayed development of the tumours. The difference between the rate of appearance of tumours in the control and interferon treated groups was highly significant ($P < 0.001$) as assessed by the log-rank test [15].

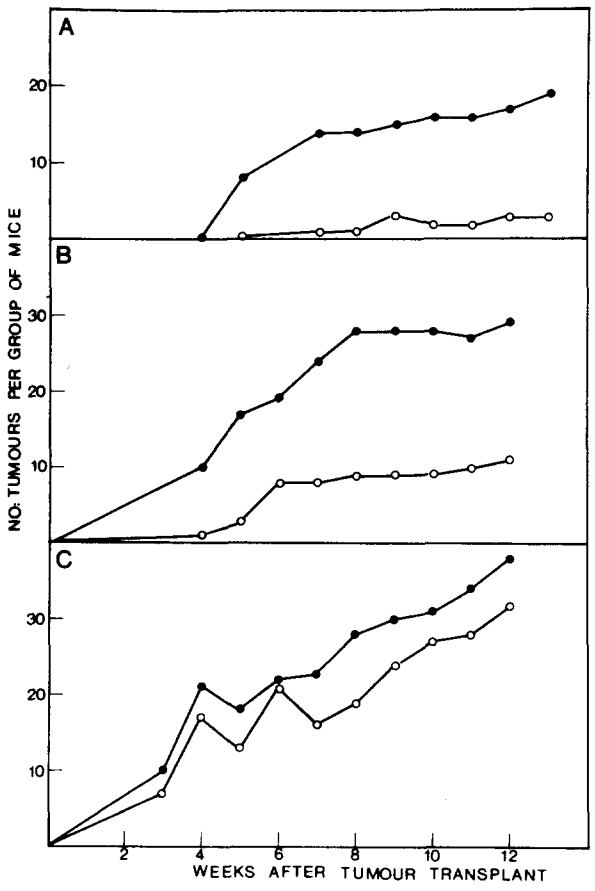

Fig. 1. *The effect of human lymphoblastoid interferon on the development of breast cancer xenografts in nude mice. A = Tumour* 630, *B = Tumour* 534, *C = Tumour* 236.—●— *control group, injected subcutaneously throughout experiment with* 50 µl *PBS* (*B*) *or* 50 µl *PBS* + 1% *FCS* (*A*, *C*),—○— *interferon treated group, injected with* 50 µl *PBS* + 1% *FCS containing* 10^4U (*B*) *or* 2×10^4U (*A*, *C*) *of human lymphoblastoid interferon. (See Methods.)*

A third experiment was carried out with tumour 630 at passage 6 to look at the effect of a potent interferon antiserum on the tumour inhibition by interferon. Twelve were divided into 2 groups of 6 mice and injected daily with 2×10^4U of interferon 580/22, or 2×10^4U of interferon completely neutralised by a 1 hr preincubation at 37°C with anti-interferon serum. This neutralisation was confirmed using the antiviral bio-assay. Mice were treated for 10 weeks, and the experiment terminated at 12 weeks. Figure 2 shows that interferon treatment completely inhibited the development of tumours and that preincubation of the interferon with the antiserum abolished this effect. The mean weight of tumours in the neutralised interferon group was 1.38 ± 20 g, which was similar to the weight of tumours in the control group for the tumour 630 experiment shown in Table 1A (1.24 ± 0.76 g).

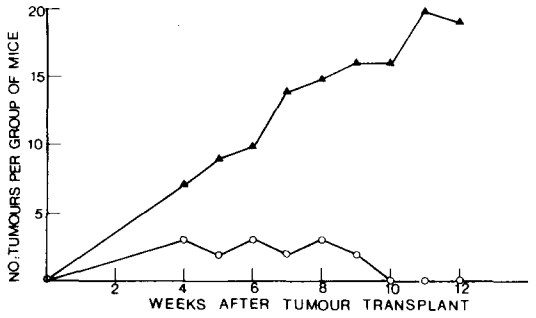

Fig. 2. Abolition of the tumour inhibitory effect of interferon by an anti-interferon serum (tumour 630).—○—Interferon treated group, injected subcutaneously throughout the experiment with 50 μl PBS+1% FCS containing 2×10^4U human lymphoblastoid interferon,—▲—neutralised interferon group, injected subcutaneously with 2×10^4U human lymphoblastoid interferon that had been completely neutralised by preincubation with antiserum to the interferon. (See Methods.)

Tumour 534

Tumour 534 at passage 4 was transplanted into 2 groups of 9 mice, which were then injected daily with 1×10^4U of interferon 580/22 or PBS. This dose had a strong inhibitory effect on the growth and development of tumours as shown in Fig. 1B and Table 1B. The difference between the rate of appearance of tumours in the control and interferon groups was statistically significant ($P < 0.005$) as assessed by the log-rank test [15]. Thus the total number of tumours that developed over a 12 week period was reduced by 62% and both the mean weight of tumour per mouse and the mean weight of the individual tumours was lowered by interferon treatment, although these differences were not statistically significant. Inhibition of growth of tumour 534 was also abrogated by preincubation with anti-interferon serum (data not shown).

Tumour 236

Two groups of 8 mice were inoculated with tumour 236 at passage 5 and then given 2×10^4U of interferon 580/22, or PBS+1% FCS daily for 12 weeks. During the first 2 weeks of the experiment one of the control mice died due to an accident. As illustrated in Fig. 1C and Table 1C, 7 mice in the control group developed 38 tumours and 8 mice in the interferon treated group developed 32 tumours. There was, however, no difference in tumour weight between control mice and interferon-treated mice and it was concluded that this tumour was insensitive to the inhibitory effects of this concentration of interferon.

DISCUSSION

Thus our results show that 2 out of the 3 human breast cancer xenografts were very sensitive to the growth inhibitory effects of lymphoblastoid interferon. Two previous reports concerning the effect of interferon on human tumours in nude mice have been less encouraging [16, 17]. However, these groups used cell lines which grew more rapidly in nude mice than the tumours described here and one group gave less frequent doses of interferon [16]. While the use of cell lines as xenografts allows a more quantitative evaluation of growth inhibition, it may be important to use cell lines derived from primary tumours at an early stage to reduce the possibility of selection of atypical cells. The lack of response of tumour 236 to interferon may be due to selection of a resistant cell type, or it could reflect an inherent resistance of cells the original tumour. Variable inhibitory effects of human interferon on the *in vitro* growth of primary human myelogenous leukaemia cells has already been observed [18] and a similar variation in sensitivity may occur among breast carcinomas.

In addition to directly inhibiting cell growth, interferons have been shown to have immune regulatory functions and to enhance the expression of surface antigens (for reviews see refs. [2], [4] and [5]). It is likely that these functions of interferon contribute to its anti-tumour effect since mouse leukaemia cells resistant to the growth inhibitory effect *in vitro* are sensitive when grown as tumours *in vivo* [19]. In examining the effect of human interferon on human tumours grown as xenografts in the nude mouse, we are almost certainly measuring a growth inhibitory effect since human interferon would not be expected to directly influence the immune response of the murine host. It is possible, therefore, that the anti-tumour effect of interferon against human tumours might well be more marked in the natural host where other cells such as those of the immune sytem may play an important role in interferon mediated tumour suppression.

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