Human tumour xenografts growing in immunodeficient mice: a useful model for assessing chemotherapeutic agents in bronchial carcinoma

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ABSTRACT Xenografts from eight human bronchial carcinomas have been established in CBA/Lac mice rendered immunodeficient by neonatal thymectomy followed three weeks later by whole body irradiation (7.35 Gy (735 rads)) after a priming dose of cytosine arabinoside. Growth rates of individual tumour lines remain constant and the histological and chromosomal characteristics of the original tumour are maintained through multiple serial passages over many months. With specific growth delay as the principal end point this system may be used to assess the response of histologically different lung tumours to chemotherapeutic agents.

Chemotherapy is now widely used for palliation in most patients with small cell lung tumours[1] and in minority of those with inoperable non-small cell malignancies.[2] The great variation in the response of individuals to the currently used drugs and the need to develop more effective agents, particularly for non-small cell tumours, has led to much interest in methods of assessing the efficiency of anticancer treatments in the laboratory.[3] The success of in vitro testing of bacterial sensitivities to antibiotics led early workers to attempt to evaluate the effects of anti-cancer drugs on malignant cells growing in tissue culture and to correlate the results with the clinical response seen in the patient.[4][5] The results were disappointing,[3] although the development of newer clonogenic assays appeared more encouraging.[6][7]

Various animal models have been used in the development and testing of new anticancer agents. Transplantable rodent tumours, such as the Lewis lung carcinoma, have proved of little value as appreciable differences in sensitivity between these and human tumours were found. In an attempt to overcome this problem many workers have grown human tumours either in congenitally athymic "nude" mice[8][9] or in rodents rendered immunodeficient by various techniques.[10][11] We have used such a system and find it a useful animal model for assessing the activity of anticancer agents in bronchial carcinoma.

Methods

PREPARATION OF IMMUNOSUPPRESSED MICE
Thymectomy was performed by retrosternal aspiration[12] on 3 week old male and female CBA/Lac mice. Three weeks later they underwent whole body x irradiation (7.35 Gy (735 rads)) via a 250 KVp, 15 mA source with a Thoraeus Ii filter at 0.37 Gy/min.
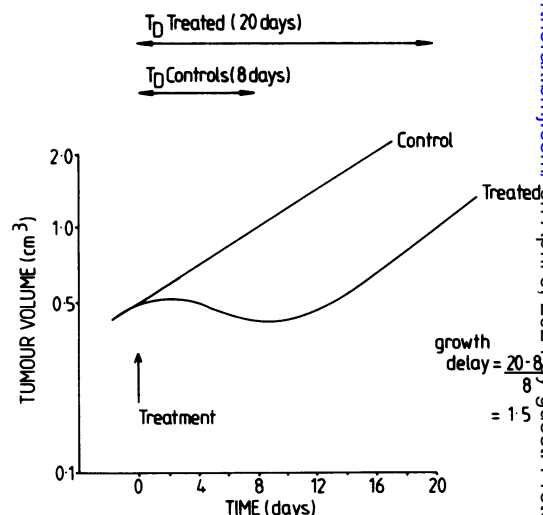

Fig 1 *Hypothetical chemotherapeutic experiment showing growth delay in treated tumours. T_D—mean tumour doubling time.*

Characteristics of established xenografts

Tumour type	Xenograft	Source	Doubling time (days)	Passage No (May 1985)	Take rate (%)
Squamous	NX002	Endobronchial biopsy	11–14	12	70
Squamous	CX108	1° resection	12–14	8	60
Squamous	CX133	1° resection	10–14	7	56
Adenocarcinoma	CX117	1° resection	10–12	10	45
Adenocarcinoma and squamous	CX143	1° resection	14–19	9	75
Large cell	WX321	Endobronchial biopsy	12	2	25
Small cell	NX004	Skin metastases	12–15	2	30
Small cell	WX322	Skin metastases	16	1	35

± 2%). Forty eight hours before irradiation a "priming" dose of cytosine arabinoside, 200 mg/kg intraperitoneally, was administered to protect the bone marrow and gastrointestinal tract from lethal radiation toxicity.[10 13] The mice were housed in a separate room in the animal unit but specific pathogen free conditions were not required. Autoclaved standard animal boxes and bedding were used. Neomycin and terramycin were added to the drinking water for 14 days after irradiation to prevent septicaemia from commensal gut flora.

PREPARATION OF XENOGRAFTS
Bronchial tumour tissue was obtained from three sources: (*a*) primary tumours resected at thoracotomy; (*b*) endobronchial biopsy specimens taken at diagnostic bronchoscopy; (*c*) subcutaneous metastatic deposits. All necrotic and non-tumour tissue was removed and 8 mm³ fragments of apparently viable tumour tissue were prepared by dissection. These were implanted without delay bilaterally into the flanks of immunosuppressed mice in the seven days after irradiation. After progressive growth to 0.3–1.0 cm³ the xenograft was either used in a chemotherapeutic experiment or passaged into a further generation of immunosuppressed animals. The histological appearance of each tumour line was checked at each passage and compared with the original specimen. The take rate of each tumour was calculated as the number of implants reaching palpable size expressed as a percentage of the number of tumour fragments implanted.

CHEMOTHERAPEUTIC EXPERIMENTS
Xenografts were measured three times weekly with callipers and serial volumes were calculated on the assumption that the tumour was ellipsoid in shape from the equation

$$\text{Volume} = \frac{\pi \times Dd^2}{6},$$

where D = largest diameter and d = smallest diameter.

Xenografts attaining a volume of 0.3–1.0 cm³ were used for drug testing. Comparable groups of 6–10 tumours were assembled and randomly allocated to either the treatment or the control group. Drugs were then administered at their maximum tolerated dose and calliper measurements continued until tumours had doubled in volume. The mean tumour doubling time (T_D) for each group could then be calculated. The overall response of xenografts to treatment was assessed by measuring the specific tumour growth delay[14] (SGD) (fig 1), where

$$\text{SGD} = \frac{T_D \text{(treated)} - T_D \text{(control)}}{T_D \text{(control)}}.$$

The specific grown delay represents the number of tumour doubling times delayed by the treatment and allows comparisons to be made between drugs in tumours of different growth rates.
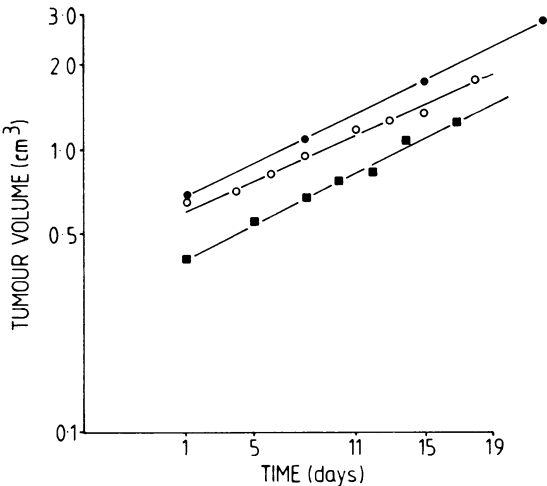

Fig 2 *Growth rates of adenocarcinoma CX117 at different passages.* ● *6th passage (6 tumours measured);* ○ *8th passage (5 tumours);* ■ *9th passage (8 tumours).*
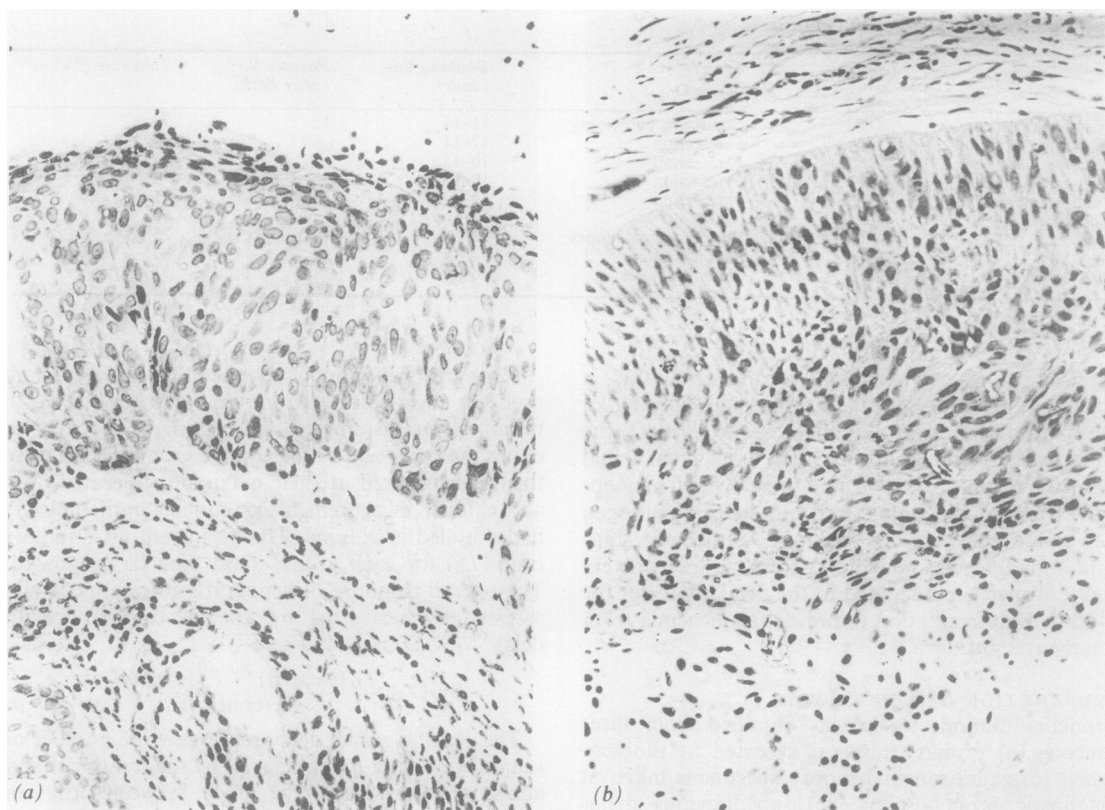
Fig 3 *Histological appearance of xenograft CX133, a moderately differentiated squamous carcinoma: (a) the original resected specimen (1982); (b) after the sixth passage (1984), showing little change in tumour morphology. (Haematoxylin and eosin, × 250.)*

Results

Using the above techniques we have established eight bronchial tumours in immunodeficient mice (table) from about 25 different patients. Take rates varied with histological type and increased with the number of times the tumour had been passaged. The growth rates of tumours remained fairly constant throughout repeated passages (fig 2), with doubling times varying from 10 to 19 days. Histological examination performed at each passage showed little change from the original donor tumour (fig 3). Some tumours became more differentiated with passaging and others more anaplastic but changes overall were small. Chromosome studies performed after the fifth passage confirmed that all tumours maintained a human karyotype.

Figure 4 shows a typical chemotherapeutic experiment, in which the effect of the nitrosourea lomustine (CCNU) on the adenocarcinoma xenograft CX117 was assessed. Control tumours grew steadily with a mean doubling time of 11.1 days. Lomustine (70 mg/kg) administered orally on day 1 caused a retardation and in many cases a temporary regression in the growth of treated xenografts. The mean T_D for the lomustine treated group was 24.4 days, giving a specific growth delay of 1.2. This figure could then be used to rank the potency of other anticancer drugs for this tumour and to compare the activities of lomustine for different tumour types. For example, cisplatin gave a specific growth delay of 0.87 in this tumour and lomustine caused complete regression of the small cell tumour NX004.

Discussion

Human bronchial carcinomas have been shown to grow successfully in nude or immunosuppressed mice
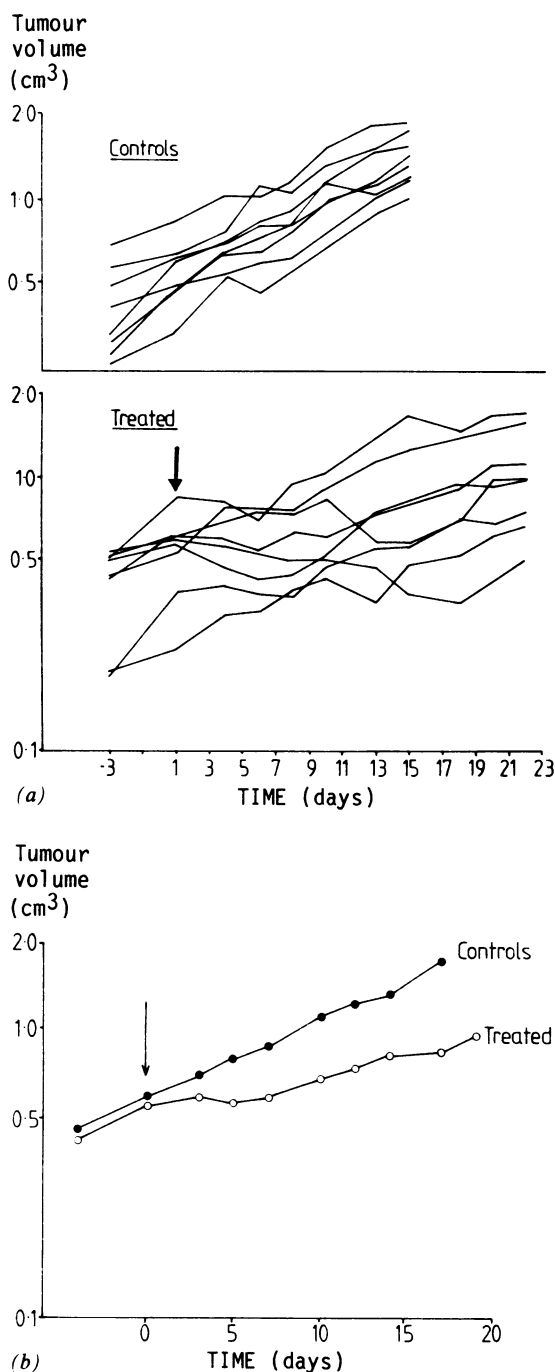
(a)

(b)

Fig 4 *Effect of lomustine 70 mg/kg (arrows) on xenograft CX117, showing delay of growth by comparison with the control group: (a) individual data from eight tumours in each group; (b) mean results from treated and control group.*

with take rates of about 40–80%, depending on the histological type of the tumour and the kind of animal model chosen.[9] [15–17] We have established several bronchial tumours in immunodeficient mice and find this a useful animal model for assessing anticancer agents. With specific growth delay as the end point of effect the potency of a single agent or drug combination can be assessed in tumours of different histological type and the sensitivity of a certain tumour to different therapeutic agents can be evaluated.

Clearly the validity of such a system depends on proof that the effect of a particular agent on a xenograft mirrors the response of the tumour in the original patient. In a direct patient-xenograft comparison Shorthouse and colleagues[18] [19] showed that the good clinical response of small cell tumours was maintained when the same tumours were treated in immunosuppressed mice and the poor clinical response of non-small cell tumours was reflected in resistant xenografts. This encouraging correlation between the responses of xenograft and donor patient has also been shown in melanoma[20] and colonic cancers.[21]

The tumour doubling times of our xenografts vary from 10 to 19 days, which compares favourably with previously reported series of bronchial carcinoma xenografts.[9] [15] [16] Surprisingly, like Steel *et al*[15] we found the small cell tumours grew at a slower rate than other types, although their doubling times are likely to shorten with subsequent passage.[9] [15]

A potential problem with assessing tumours growing in animals rendered artificially immunodeficient is the tendency of the host response to return and influence the growth of xenografts.[10] Since, however, human tumours grow at a much faster rate in mice, most experiments can be completed in about 10 weeks from transplantation, and the period that an animal remains adequately immunosuppressed can be extended to over six months when high doses of irradiation are used.[22] The use of nude mice may help to overcome this difficulty but these animals are costly, require elaborate husbandry, and are also capable of mounting a host response.[23] On balance we have found thymectomised irradiated mice cheaper and easier to use than nude animals.[24]

The time taken for xenografts to grow to an adequate size for testing makes the use of this system for predicting the drug sensitivity of individual tumours in the clinical setting impractical. As a model for assessing new anticancer agents in bronchial carcinoma, however, it is inexpensive and easy to establish while, as Shorthouse and colleagues have shown,[18] [19] capable of producing clinically valid results.

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