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THE THERAPEUTIC RESPONSE OF BRONCHIAL CARCINOMA XENOGRAFTS: A DIRECT PATIENT-XENOGRAFT COMPARISON

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Summary.—The chemotherapeutic response of a series of patients with bronchial carcinoma has been compared with the response of their xenografts established in immune-suppressed CBA/lac mice. Xenograft response was assessed by the *in situ* endpoint of growth delay in subcutaneous tumours. Histology and chromosome analysis indicated that human characteristics were retained in the xenografts. 49 xenograft lines were attempted: 15/18 oat cell, 11/17 squamous, 6/8 large cell anaplastic and 6/6 adenocarcinomas were successfully established. 14 Lines (28%) were available (August 1979) for direct comparison of xenograft and donor patient response to the same chemotherapeutic agents. A positive correlation was found and provides support for the chemotherapeutic validity of xenografts.

THE AIM of this study was to compare the xenograft/donor patient responses to the same agents in a series of bronchial carcinomas. This category of tumour was chosen because in the clinic there is a wide spectrum of response to chemotherapy which may frequently be objectively assessed.

MATERIALS AND METHODS

Xenografts were established by implanting 2–3 mm tumour fragments s.c. over the posterior rib cage of mice that had been immune-suppressed by thymectomy, Ara-C treatment and whole-body irradiation (Steel *et al.*, 1978). Groups of tumours were selected for chemotherapy when their mean volume was 0·2–0·5 cm^3, calculated as $\pi/6 \times (\text{mean diameter})^3$. They were randomly allocated to treatment and control groups and therapeutic response was measured by the *in situ* endpoint of specific growth delay. This was determined by finding the difference between the median time to double in volume of control and treated groups of tumour, and dividing the result by the median doubling time of the control tumours (Kopper & Steel, 1975). This gave an estimate of growth delay in multiples of the doubling time of untreated tumours. All experiments were performed within the first 5 passages.

Clinical response was assessed by recording the size of measurable lesions and by determination of biochemical parameters. Clinical response was documented by JFS and xenograft responses were studied by AJS; these were done independently.

In order to parallel the clinical situation each xenograft line received the agents that were administered to the respective donor patient. Although patients received repeated cycles of drug combinations or single agents, the mice were limited to single cycles. Schedules of drug administration within a cycle were similar. When drug combinations were used, the dose ratio of component agents in patients was strictly maintained in the mouse studies. There is no universally satisfactory basis for converting human to murine doses. The policy that we adopted was to employ the maximum tolerated dose (MTD) in the mouse (Freireich *et al.*, 1966) as determined from preliminary toxicity studies.

All agents were given by the intraperitoneal route in a volume of 0·01 ml/g of mouse body weight. Methotrexate, as a single agent or in combination, was given in 3 divided doses over 24 h, attempting to mimic a 24h infusion in donor patients. No folinic acid rescue technique was applied in the mice. Iphosphamide was administered once daily for 3 days. Vincristine was given as a single injection on Days 1 and 5. Other agents were given as single doses. All drugs were prepared in aqueous solution with the exception of CCNU (10% dimethylsulphoxide in 5% Tween 80 in normal saline).

RESULTS

Bronchial carcinomas from 49 patients were xenografted and the success rates in each histological category are shown in Table I. The criterion for a xenograft take was a graft progressively growing beyond 0·2 cm^3. Large cell anaplastic and adenocarcinoma produced a larger proportion of successful grafts than oat cell or squamous cell carcinoma ($P < 0·01$). However, no significant variation was found among the subgroups with respect to the number of patients from whom at least a single successful graft was obtained, because even single takes from multiple implants were usually sufficient to produce xenograft lines.

Chemotherapeutic responses

The results of treatment of patients and xenografts are summarized in Table II. The logistic problems faced in this study were that only 28/49 (57%) patients biopsied for xenografting received chemotherapy. Objective assessment of response was made in 22 (45%) but it was only possible to successfully establish xenograft lines for comparative experimental

TABLE I.—*Xenograft take rates of human bronchial carcinoma in immune-suppressed mice*

Tumour	No. patients attempted	Mean no. implants per patient	No successful grafts*	Proportion of successful grafts	Graft success in subsequent passage
Adenocarcinoma	6	12	6	34/70	6
Oat cell	18	12	15	62/212	11
Large cell	8	7	6	35/54	4
Squamous	17	7	11	38/118	5

* Established in one or more implants.

TABLE II.—*Comparison of chemotherapeutic response of bronchial carcinoma xenografts and donor patients*

Tumour	Source	Xenograft designation	Treatment*	Patient response	Xenograft response†
Oat cell	Lymph node	HX88	Iphos	+	Cure
	Skin 2°	HX81	MCC	+	Cure
	Lymph node	HX89	MCC	+	Cure
	Lymph node	HX96	MCC	+	Cure
	Skin 2°	HX71	MCC	+§	Cure
	Skin 2°	HX72	MCC	–	2·4
Squamous	Lymph node	HX79	Mtx	–	0·7
	Lymph node	HX64	VBMF	–	0·7‡
	Lymph node	HX97	Mtx	–	0
Adenocarcinoma	Skin 2°	HX83	CAF	–	1·3
	Skin 2°	HX70	CF	–	0·2
			CAF	–	0
	Lymph node	HX87	CAF	–	0
			Mtx	–	0·3
	Skin 2°	HX94	MCC	–	0·4
Large cell	Lymph node	HX82	CAF	–	1·7

* Single agent and combination chemotherapy abbreviations:
MCC—Methotrexate/Cyclophosphamide/CCNU
VBMF—Vincristine/Bleomycin/Methotrexate/5-FU
CAF—Cyclophosphamide/Adriamycin/5-FU
CF—Cyclophosphamide/5-FU
Mtx—Methotrexate
Iphos—Iphosphamide

† Measured in terms of specific growth delay at maximum tolerated dose

‡ Xenograft growth delay at maximum tolerated dose of single agents only.

§ Patient exhibited a differential response.

chemotherapy in 17 (34%). Results of 14 are included in this report. Five (19%) other patients died after chemotherapy before any objective response could be made. One received adjuvant chemotherapy alone and 5 (10%) died prior to planned chemotherapy. Fourteen further patients from whom xenografts were successfully established received no chemotherapy.

Objective and quantitative assessment of chemotherapeutic response in donor patients was by serial measurement at various metastatic sites. Superficial metastases in the skin or supraclavicular lymphadenopathy could be more accurately measured than lesions at other sites, *e.g.* hilar lymph nodes. Although the precision with which clinical response could be documented varied considerably from one patient to another there remained a clear difference between responders and non-responders.

Oat cell carcinoma.—As indicated in Table II 4 untreated patients with oat cell carcinoma received MCC and in each case they demonstrated complete regression of their disease. A fifth untreated patient received iphosphamide which also induced complete remission. One oat cell carcinoma patient had been previously treated with MCC before the xenograft biopsy was taken (HX72) and her disease responded poorly to retreatment. The xenografts of all except the latter patient were completely cured by MCC or iphosphamide treatment; xenografts from HX72 were not cured and showed a median specific growth delay of only 2·4 doubling times.

Non-oat bronchial carcinomas.—In 3 cases of squamous cell carcinoma, 4 cases of adenocarcinoma and one case of large cell anaplastic carcinoma clinical responses to combination chemotherapy were poor. Xenograft responses in most cases were barely detectable and only in 2 cases exceeded a growth delay of 1·0 volume doubling time.

Xenograft morphology

Histology and chromosome analysis indicated that human characteristics were retained in all xenograft lines. Most xenografts were hyperdiploid. The pathologic features and other aspects of xenograft tumour biology will be published elsewhere.

DISCUSSION

The chemotherapeutic validity of xenografts is supported by the results of this study, in which responses of 14 patients with bronchial carcinoma and their respective xenografts were found to correlate well. The differences observed between the chemosensitivity of most oat cell carcinomas and the resistance of large cell anaplastic, squamous and adeno-carcinomas were marked both in man and xenografts.

Within any particular histopathological category, however, rank correlations of response between patients and xenografts were difficult to determine, basically because a growth delay value could not be attributed to many patient responses. Xenograft responses to a maximum tolerated dose of drug could be quantified in terms of tumour growth delay in the more resistant tumours but not in oat cell carcinomas after complete regressions had occurred. This problem is being rectified in current experiments by treating these tumours at doses sufficiently low to allow tumour regrowth after transient regressions. Knowledge of the shape of the dose response curve allows extrapolation of the resultant growth delay to the value at the maximum tolerated dose. Additionally, this manoeuvre usefully avoids the dilemma of interpretation of tumour cures in xenografts and the extent to which they are artefacts caused by host defence mechanisms, perhaps the most serious criticism levelled at the use of immune-suppressed mice for *in situ* chemotherapeutic experiments (Kopper & Steel, 1975; Steel, 1978). Nevertheless, considerable chemosensitivity was demonstrated in 5 oat cell carcinoma xenografts with 100% tumour control at the maximum

tolerated dose of the MCC and iphosphamide regimes. Although host immunity may have contributed to some extent it is significant that: (1) 100% tumour control has been achieved at only 20% of the maximum tolerated dose in HX96; (2) regressions *with subsequent regrowth* occurred at 35% of the maximum tolerated dose in HX88 and other xenografts; (3) complete objective tumour regressions were also documented in respective donor patients.

Considerable variation of response to the MCC combination occurred in donor patients. Four with oat cell carcinoma responded well while a patient with adenocarcinoma was resistant. This spectrum of response was reproduced in the xenografts. Additionally, it is encouraging that *acquired* drug resistance in the patient appeared to be retained through 3 tumour passages in the mouse. This xenograft (HX72) was one of several lines established with some difficulty due to low take rates in the initial passages. Despite the probability of a large degree of clonal selection in adapting to the mouse environment through successive passages, the appropriate drug resistant sublines seem to have persisted. Further work is needed to verify this observation and to determine whether acquired drug resistance is lost in later passages.

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