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Letter to the Editor

Divergent Effects of Double-Stranded RNA on Growth of Rat Tumours in Syngeneic Recipients and Athymic Nude Mice*

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THE ATHYMIC nude mouse is becoming widely used to examine the influence on experimental and human xenografts of chemotherapy [1], radiotherapy [2] and immunotherapy [3, 4], the contention being that susceptibility of tumours as xenografts may be indicative of susceptibility in syngeneic transplants or, particularly with human tumours, in the autochthonous host. In these contexts, mouse and human tumour grafts in athymic mice are susceptible to systemic treatment with double-stranded RNA [5, 6] and the interpretation is that this may be due to either direct toxicity of ds-RNA for malignant cells or the anti-tumour effect of interferon induced by the agent [5, 6], although growth is not suppressed by interferon treatment alone [5, 6]. The objective of the present communication is to report that rat tumours susceptible to local ds-RNA treatment in syngeneic transplant are not susceptible when xenografted to athymic nude mice.

Sarcoma Mc7 was originally induced in this Laboratory in a female WAB/Not rat by the subcutaneous injection of 3-methylcholanthrene, and hepatoma D23 in a male rat of the same strain by oral administration of 4-dimethylaminoazobenzene. Both tumours have been routinely passaged as subcutaneous grafts in syngeneic rats [7, 8]. For part of the present work *in vitro* cultures were established and maintained in Eagle's medium supplemented with 10% calf serum.

Natural double-stranded RNA, from fungal virus, was supplied by Beecham Research Laboratories, Betchworth, Surrey, as a freeze-dried preparation (BRL 5907). The material was reconstituted in water to 5 mg/ml, sterilised by passage through a 0.22 μm Millipore® filter and stored frozen at −20°C [7].

Cells harvested from tissue culture or prepared by trypsin digestion of solid tissue were injected subcutaneously into the right flank of syngeneic rats or athymic nude mice (ONU, MRC Laboratory Animal Centre, Carshalton, Surrey) either alone or in direct admixture with ds-RNA at 50–125 μg/inoculum.

With both the hepatoma and the sarcoma, incorporation of ds-RNA into tumour cell inocula completely prevented tumour development in the rat, with both tissue cultured cells and cells prepared from solid growths. In contrast, tumour growth was totally unaffected in the athymic mouse (Table 1). For example, with the hepatoma D23, incorporation of 50 μg ds-RNA into inocula of 1×10^5 tumour cells completely prevented growth in syngeneic rats, but growth occurred in all athymic mice receiving similar inocula with up to 125 μg ds-RNA. For tests in athymic mice cells from tissue culture only were used to prevent the transfer of rat lymphoreticular cells present in digests of solid tumour tissue. A further series of tests was carried out with the tumours in syngeneic transplant in the rat to determine whether host immunosuppression

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by whole body irradiation, previously established to abrogate the induction of tumour-specific transplantation resistance [9], could influence local tumour suppression by ds-RNA [2]. The results from these studies (Table 2) show that ds-RNA controlled growth of sarcoma Mc7 and hepatoma D23 in both normal and irradiated (450R) animals.

Essentially, these studies indicate that an agent markedly tumour suppressive in syngeneic hosts is ineffective against tumour xenografted to athymic mice. Previous studies with syngeneic grafts of the rat tumours used here [7, 8] demonstrated that systemic treatment with ds-RNA was only marginally effective, in contrast to several other reports of successful control of tumour growth [10, 11]. However, local injection was markedly tumour suppressive against subcutaneous, pleural and peritoneal growths [7, 8], in keeping with observations of other workers [12–14], and consequently only this method of treatment was used in the present work.

Double-stranded RNA is cytotoxic *in vitro* for tumour cells [5, 7], and cells treated with the agent and extensively washed fail to grow *in vitro* [7]. However, direct, indiscriminate, cytotoxicity cannot wholly explain the antitumour effect of locally applied ds-RNA in syngeneic transplant, since tumours vary in susceptibility [7], and the present tests show that mixed inocula are tumorigenic in athymic mice. The implication from this is that host responses are involved, but rats rejecting tumour cells and ds-RNA are not immune to further challenge [7], implying that systemic antitumour immunity is not necessary for tumour control. This is supported by results of the present tests, where tumours were controlled in immunosuppressed rats, and suggests that local non-specific host responses may be involved. These may include local interferon production, with possible effects on the levels of Normal Killer (NK) cell activity [15], or the local activation of host macrophages [16]. The present work

Table 1. *Subcutaneous growth of rat tumour cells injected in admixture with double-stranded RNA into syngeneic rats and athymic nude mice*

Rat tumour	Mixed subcutaneous inoculum		Tumour growth in: Syngeneic rats		Athymic mouse*	
	No. cells	μg ds-RNA	Test	Control	Test	Control
Sarcoma Mc7	1×10^6	125	0/6*	6/6*	3/3	3/3
	1×10^6	125	0/4†	4/4†	—	—
Hepatoma D23	1×10^5	125	0/4†	4/4†	3/3	3/3
	1×10^5	125	0/4*	3/4*	3/3	2/2
	1×10^5	50	0/5†	5/5†	3/3	4/4

*Cells harvested from tissue culture.
†Cells prepared by trypsin digestion of solid growths in syngeneic rats.

Table 2. *Influence of whole body irradiation on growth of subcutaneous inocula of rat tumour cells in admixture with double-stranded RNA*

Tumour	Mixed subcutaneous inoculum		Tumour growth in:	
	No. cells*	μg ds-RNA	Irradiated rats†	Normal rats
Sarcoma Mc7	1×10^6	125	0/4	5/5
	1×10^6	—	5/5	6/6
Hepatoma D23	1×10^5	125	2/6	0/6
	1×10^5	—	4/4	6/6
D23	1×10^5	125	1/6	0/6
	1×10^5	—	6/6	5/5

*Cells prepared by trypsin digestion of solid growth in syngeneic rats.
†450R ^{60}Co γ-irradiation 24 hr beforehand.

indicates, however, that effective non-specific host responses cannot be generated in the athymic mouse by locally applied ds-RNA. This is in contrast to bacterial preparations (BCG and *C. parvum*) which are locally tumour suppressive against rat tumours both in syngeneic animals [17] and against rat and human tumours in athymic mice [3, 4, 18], and current evidence indicates that host phagocytic cells are involved in, if not the direct mediators of, this antitumour response [17].

Clearly the mechanism of tumour suppression by double-stranded RNA's, against both syngeneic tumours and athymic mouse xenografts, requires further investigation, but the indication from the present findings is that local tumour suppression in the syngeneic host, although not dependent upon specific immune responses, may not be reflected in the response in the athymic mouse.

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