Growth characteristics and sensitivity to levan of various spontaneous AKR lymphomas

Spontaneous tumor	Maximal size of local tumor (mm) ± SD	Latency of tumor appearance (days)		Nontreated		Levan-treated	
		Local	Inguinal lymph node	MST (days)	Surviving mice on day 200	MST (days)	Surviving mice on day 200
D	3.5 ± 2.2	64	78	109	1/10	199	5/10
E	20.0 ± 7.7	20	25	51	0/10	91	5/10
C	-	-	12	30	0/10	30	3/10
B	-	-	16	33	0/10	33	1/10

Discussion. The results presented show that in the AKR lymphoma system, spontaneous tumors may differ: 1. in their degree of malignancy; 2. in their sensitivity to drugs. We found a relationship between the degree of malignancy and the sensitivity of the tumor to the polysaccharide levan. The tumor we used in our previous studies[2,3] behaved similarly: it formed very large local tumors (up to 40 mm in diameter) and was very sensitive to levan (up to 100% cure in some experiments). Two possible explanations for the observed differences can be considered: the degree of malignancy may depend on the stage of progression in which the spontaneous tumor was at the moment at which it has been used for the 1st transfer, or, alternatively, clones of varying degrees of malignancy may arise in different individuals. The results of Rees and Westwood[8] can be regarded as evidence favoring the 1st possibility, since spontaneous carcinomata taken late after tumor inoculation showed a more rapid increase in malignancy following serial passage than young tumors. Our results support this possibility.

Tumor progression was shown to be associated with selection of more and more aggressive cells from a previously heterogeneous population. This was well demonstrated in the B16 melanoma by Fidler[9]. AKR lymphoma tumors were recently also shown to consist of antigenically heterogeneous populations with regard to antigenicity[10]. It is therefore possible that in different spontaneous tumors, different variants, perhaps antigenic, are dominant. Differences in antigenicity may actually explain differences in sensitivity to levan, since this polysaccharide was shown to act mainly by a host-induced mechanism[11,12].

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Comparison of DMSO and glycerol as cryoprotectants for ascites tumor cells[1]

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Summary. Mouse Ehrlich ascites and rat D23 ascites tumors were stored in liquid nitrogen under identical conditions for up to 3 years. Cell viability (trypan blue exclusion) and transplantability of both tumors in animals remained virtually unaffected if preserved in 10% DMSO containing medium, whereas, cells preserved in 10% glycerol failed to produce lethal tumors in rodents.

Ascitic variants of animal tumors are widely used in experimental cancer research[2,3]. Cryopreservation of cells would allow the use of original tumor material for interrelated experiments conducted over many years. It has been previously reported that the transplantability of mouse ascites tumors stored at −78 °C in 10% glycerol decreased with increasing storage time[4,5]. Much greater numbers of viable ascites cells were required to produce lethal tumors in animals, the tumor took longer to develop and the percentage of animals with tumors was between 10 and 70% compared to 100% with fresh unstored cells[5]. Shah and Dickson[6] recently described a simple long term (at least 10 years) storage method for preserving enzymatically prepared rabbit VX2 carcinoma cells at −196 °C. The tumor take, growth characteristics and antigenicity of such rabbit VX2 cells[7,8] or rat Mc7 tumor cells[9] when inoculated into animals after preservation in liquid nitrogen have remained unchanged. This method of storage at −196 °C was therefore further investigated for mouse and rat ascites tumor cells, using DMSO and glycerol as low temperature preservatives under identical conditions of storage.

Methods and materials. Ascites tumors. The Ehrlich ascites tumor, EAT (tetraploid strain; obtained from Marie Curie Memorial Foundation, Oxtend, Surrey, U.K.) was maintained by serial passage of 10^6 cells injected i.p. into 30–35 g male To Swiss mice (Tucks, Rayleigh, U.K.). This cell dose killed mice at between 14 and 18 days. Over 95% viable cells, as determined by trypan blue dye uptake, were

Effect of the method of preservation on survival of mouse EAT and rat D23 ascites tumor cells

Tumor type	Storage time at −196 °C (days)	Method of preservation[a] Glycerol % unstained cells[b]	No. with tumor/total[c]	DMSO % unstained cells[b]	No. with tumor/total[c]
EAT	0	97	2/2	97	2/2
	7	60	1/2	91	2/2
	42	24	0/2	90	2/2
	176	<1	0/2	88	2/2
	723	<1	0/3	82	3/3
	1080	<1	0/4	84	4/4
D23	0	96	2/2	96	2/2
	7	53	1/2	90	2/2
	42	18	0/2	87	2/2
	176	<1	0/2	90	2/2
	723	<1	0/2	83	3/3
	1080	<1	0/4	81	4/4

[a]Storage medium contained 1% BSA and 10% glycerol or 10% DMSO. Cells were initially stored at −70 °C for 20 h and then transferred to liquid N_2 tank at −196 °C. [b]Trypan blue stain. Unstained cells are presumably viable. Both EAT and D23 cells in glycerol or DMSO containing medium were freezed or thawed side by side under identical conditions. No repeated freezing and thawing of the same sample was performed. [c]Animals were held for up to 40 days, and results are recorded as the number of rodents developing tumors over the number inoculated.

usually harvested at 7–8 days after i.p. transplantation. Only non-hemorrhagic ascites tumors were used for freeze preservation. An ascitic variant of syngeneic hepatocellular carcinoma D23 As[3] (obtained from Cancer Research Campaign Laboratories, Nottingham, U.K.), was maintained by serial i.p. transplantation of 10^7 cells in male WAB/Not rats. The rats died at 18–20 days after injection of this cell dose. Cells were harvested at 7–8 days when the viability was more than 95%. Both types of ascites tumor cells were collected by centrifugation, and washed twice with Waymouth culture medium (Difco 5043-37-5 TC Medium MB 752/1 Dried) containing 1% bovine serum albumin (BSA: Sigma Chemical Co., Albumin bovine, fraction V). The following method was employed for storing cells.

Freezing in liquid nitrogen (−196 °C): The tumor cells were stored in Waymouth medium containing 1% BSA and 10% glycerol or 10% dimethyl sulfoxide (DMSO). The sealed borosilicate glass ampules each containing 7.5×10^6 cells in 1 ml of the above medium after 10 min incubation at room temperature were initially placed in a deep freeze at −70 °C for 20 h. The ampules were then transferred into a liquid nitrogen storage tank at −196 °C. When required, the ampules were rapidly thawed in a waterbath at 37 °C. Cells from each ampule were diluted with 10 ml of BSA-containing medium and washed once with BSA free Waymouth medium.

Results and discussion. The survival of both types of ascities tumor cells when preserved in 10% DMSO containing medium, remained virtually unaltered for up to 3 years after storage. On the other hand, the viability of cells in 10% glycerol containing medium decreased to below 25% within the 1st 6 weeks of storage at −196 °C (table). Tumor suspensions recovered from glycerol-containing medium always showed cells with displaced nuclei and wide spread cell lysis; this did not occur when DMSO was used as a preservative. Also, tumor cells that were stored in glycerol medium consistently failed to produce tumors in animals after 6 weeks of storage. The rate of tumor production by EAT and D23 cells in animals after inoculation of fresh cells or with cells preserved for up to 3 years in DMSO-containing medium was similar. That is, mice died at between 14 and 18 days after inoculation of 10^6 viable EAT cells and the rats died at 18–20 days after 10^7 D23 tumor implant. Altogether, 59 animals were used for testing frozen tumor cells taken at different time points during the 3-year period (table).

The use of medium containing albumin and DMSO and the storage temperature of −196 °C may account for the superior results obtained in the present work. Hewitt and Blake[10] have also reported that WHT ascites lymphoma can be well preserved at −196 °C for up to 9 years in Tyrode solution containing 10% DMSO and 10% mouse serum. These workers observed that the TCD_{50} (tumor cell dose required to kill 50% animals) for mice remained unaltered with cells before, after, and after single post-storage passage. The survival of ascites tumor cells in 10% glycerol containing medium at either −78 °C[4,5] or at −196 °C (present work) decrease sharply with time. Our results suggest that it is important to determine the optimal conditions of storage for the tumor under study employing both DMSO and glycerol as preservatives. In our previous work, the enzymatically disaggregated rabbit VX2 tumor cells were found to survive better in 10% glycerol than in 10% DMSO at −196 °C[6]. These findings may be partly attributed to the difference in the removal of cryoprotectants by various tumor cell types during the rewarming in water bath at 37 °C. Complete removal of glycerol or DMSO from stored cells is necessary due to the possible toxicity at normal body temperature[11].

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