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Spontaneous AKR lymphomas differ in their degree of malignancy and sensitivity to the polysaccharide levan

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Summary. Spontaneous AKR lymphomas differ in their biological behavior as judged by formation of local tumors at the site of inoculation, latency in the appearance of both local and distant tumors and mean survival time of the mice. Spontaneous AKR lymphomas differ markedly in their sensitivity to levan (polyfructose). An inverse correlation was observed between the degree of malignancy and sensitivity to levan.

AKR lymphoma has been shown to respond to therapy by the polysaccharide levan[2,3]. We have also observed that this tumor loses its sensitivity to levan following serial passages[3,4]. Serial passages also caused an increase in the malignant behavior of the tumor, a feature which was also found by another group[5]. The loss of sensitivity to levan seemed to be related to the degree of malignancy.

In the present study we investigated the malignant behavior and sensitivity to levan treatment of various spontaneous AKR tumors.

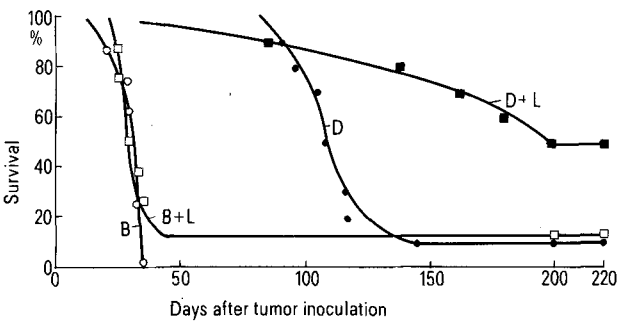
Materials and methods. Experiments were performed with AKR/Cu male mice, 6–10 weeks old, obtained from the Weizmann Institute, Rehovoth. Spontaneous tumors were obtained from aged AKR mice. Tumor cell suspensions were separately prepared from 4 spontaneous tumors. The cell suspensions were prepared from mesenteric lymph nodes as previously described[2]. 5×10^4 tumor cells from each spontaneous tumor were inoculated s.c. in the back of groups of 20 mice. Ten mice of each group were treated locally with levan obtained from the Department of Biological Chemistry, Technical Unit, the Hebrew University, Jerusalem. Levan was prepared from *Aerobacter levanicum* according to Hestrin et al.[6] and solutions were prepared according to Shilo et al.[7]. Daily injections of 10 mg in 0.2 ml saline per mouse, were begun on the day of tumor inoculation and continued throughout the experiment.

Local and distant (inguinal lymph nodes) growth was assessed by palpation every 2–3 days. Local growth, when present, was measured. Mortality of mice was recorded daily.

Results. The table shows the growth characteristics and sensitivity to levan of 4 spontaneous tumors, D, E, C and B, in the 1st transfer. The survival curves of untreated and levan-treated mice of tumors B and D are shown in the figure. The various spontaneous tumors differed from one another in the degree of malignancy as gauged by formation of local tumors at the site of inoculation, latency of local and distant tumor appearance, and mean survival time (MST).

The less malignant tumor was D; the most malignant were B and C. The latency of tumor appearance and the MST were positively correlated. Tumors D and E formed local growths at the site of inoculation, while tumors B and C did not. Although the capacity to form local tumors denotes a lesser degree of malignancy, no strict correlation was observed between the size of local tumors and MST.

The B tumor was much more aggressive than the D tumor; 100% of mice were already dead on day 35 in tumor B, while tumor D killed 9/10 mice only on day 145. Sensitivity to levan treatment was inversely proportional to the degree of malignancy of the tumor. While tumor D was effectively inhibited, tumor B was practically insensitive to the polysaccharide; in animals bearing the less malignant tumor D, 5/10 treated mice survived for 199 days, whereas in those injected with the malignant tumor B, only 1/10 remained alive by the 43rd day.



Survival of AKR mice inoculated with B and D tumors, treated or untreated by levan. 5×10^4 AKR lymphoma cells of the 2 spontaneous tumors B and D were inoculated s.c. in the back of 18 or 20 AKR mice. Eight animals inoculated with tumor B and 10 inoculated with D were not further treated. Similar numbers of mice were treated with levan. Tumor B: untreated, ○; treated with levan, □; tumor D: untreated, ●; treated with levan, ■.

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