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Effects of High-Molecular Levan on the Growth and Spread of Lymphoma in AKR Mice[1]

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SUMMARY

Levan was shown to inhibit lymphoma development in AKR mice. Growth of tumor was inhibited at the site of injection and in metastases. Levan caused a decrease in the incidence of tumors, and it reduced pleomorphism, mitoses, and invasiveness of tumors in comparison to nonlevanized mice. Evidence of tumor cell destruction was observed in levanized mice. The effect of levan on tumor development was dose dependent.

INTRODUCTION

Native high-molecular levan is known to block the passage of colloidal substances, including plasma proteins, across the walls of vascular channels (3, 7). Large doses of levan block the passage of polymorphonuclear granulocytes into tissue that has been inoculated by pyogenic bacteria and thereby inhibit the acute inflammatory response (19). Also, wound healing and formation of granulation tissue are inhibited by this heavy neutral polysaccharide (21). The polysaccharide appears to act mainly by coating the inner surface of endothelia and changing the permeability of this layer. It has been shown that continuous administration of levan is associated with transfer of the polysaccharide across the vessel wall and its deposition in phagocytic cells and in the connective tissue ground substance (20, 21).

The blocking of transendothelial passage and the change in the microenvironment of individual cells produced by continuous administration of levan might affect the growth and spread of malignant tumors. This study reports experiments performed to test this hypothesis.

MATERIALS AND METHODS

Mice and Tumor. Six-week-old AKR/Cu male mice of the Weizmann Institute stock were used. The AKR lymphoma was maintained by intrastrain i.p. passages of spleen cell suspensions (about 10^7 cells).

Levan. Native *Aerobacter* levan prepared according to the method of Hestrin *et al.* (12) was purchased at the Department of Biological Chemistry, Technical Unit, The Hebrew University of Jerusalem. Its molecular weight was approximately 20×10^6. A 5% solution in 0.85% NaCl solution was prepared according to the method of Shilo *et al.* (19).

Levan was administered by daily i.p. injections beginning 1 day before tumor inoculation. Groups of animals received daily doses ranging from 5 to 25 mg levan. To minimize toxicity, 3 preparatory injections of 5 or 10 mg were administered at Days 6, 4, and 2 before tumor injection.

Tumor Cell Inoculation. Tumor cell suspensions from mesenteric lymph nodes suspended in Dulbecco-Vogt medium were filtered through several layers of gauze to remove debris. Cells were counted in a hemacytometer with trypan blue exclusion as a criterion of vitality. All the steps were done at 4°. The freshly prepared suspension (10^5 cells) was inoculated s.c. into the backs of the animals.

Evaluation of Tumor Development. Tumor development was evaluated by the following criteria: (*a*) incidence of tumors, local and metastatic, by palpation and at autopsy; (*b*) size of tumors at site of injection and in metastases as a function of time after inoculation was followed by palpation. Size of tumors was expressed in arbitrary units from 0 to 8, with each unit roughly corresponding to 1 mm in diameter; (*c*) at autopsy, the size of tumors was determined by weight; (*d*) full autopsies were performed in 4 animals for each level of levan and in 4 nonlevanized mice. All these mice were inoculated s.c. with 10^5 cells. Histological sections were prepared from the primary tumor and surrounding tissues, lymph nodes, spleen, and liver. Hematoxylin and eosin and the periodic acid-Schiff procedures were used for all sections.

RESULTS

Inhibitory Effect of Levan on the Development of Primary Tumors. Continuous administration of levan decreased the incidence of tumors at the injection site. The inhibition of tumor development was dependent on the dose of levan. Repeated experiments have shown that the highest doses of levan (25 mg/day) almost completely inhibited the growth of primary tumors. Lower levan doses were progressively less effective, but still effected a significant delay of growth.

Table 1 shows the result of a typical experiment when at

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28 days no tumors were palpated in animals that had received 25 mg levan daily, while tumors occurred in 63% of the nonlevanized mice. The average weights of the animals in this experiment were 28.4 g for nonlevanized mice and 26.8, 26.1, and 29.0 g for mice treated with 5, 10, and 25 mg of levan, respectively. An increase of the inoculum of tumor cells to 10^6 instead of the usual 10^5 yielded similar results.

The development with time of primary tumors was also inhibited by levan. The effect depended on the dose of levan. Chart 1 illustrates the kinetics of tumor growth with time as a function of the amount of levan administered. In nonlevanized animals, the incidence of large tumors increased rapidly with time. In levanized mice, the increase in size proceeded at a slower rate with increasing doses of levan, being almost nil with 25 mg/day. In some instances, apparent regression of previously palpable tumors was observed.

Table 1

Incidence of local tumors in AKR mice treated with increasing concentrations of levan 28 days after tumor injection

Each mouse received an injection of 10^5 tumor cells.

Amount of levan injected daily (mg)	Tumor incidence[a]	% incidence	p
	12/19	63	
5	6/25	24	<0.02
10	2/27	7	<0.05
25	0/17	0	<0.001

[a] No. of mice developing local tumors/number of mice treated.

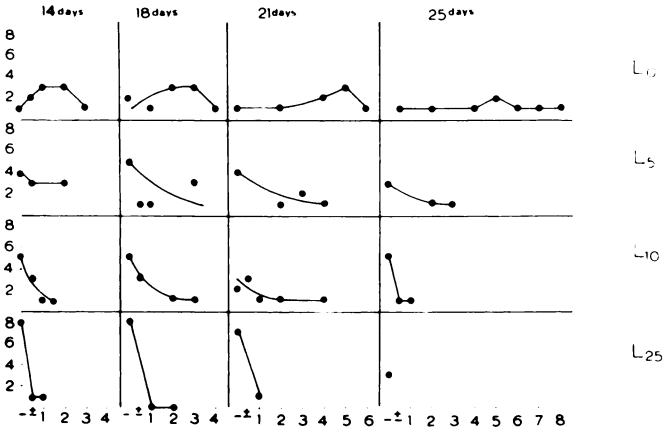

Chart 1. Tumor growth as a function of time in mice given different amounts of levan. *Abscissa*, size of tumors in arbitrary units (each unit = approximately 1 mm in tumor diameter). *Left ordinate*, number of animals with tumors of any given size. *Top*, days after inoculation of tumor cells. *Right ordinate*, dose of levan administered daily (in mg).

A more objective evaluation of the effect of levan on tumor growth was obtained by weighing tumors from animals killed 34 days after inoculation. Table 2, Column 1 presents the mean weight of tumors in animals treated with varying doses of levan. The data indicate that the effect of levan on tumor size is statistically significant, which confirms the palpatory findings.

Inhibition of Metastatic Tumor Growth by Levan. Metastatic spread was evaluated only in autopsied animals. The effect of levan on metastases was similar to that observed in the primary tumors, as can be seen in Table 2.

Histological study of lymph nodes and spleens showed that the changes induced by levan were similar to those observed in the primary tumors.

Survival of Tumor-bearing Animals. Administration of levan shortened the life-span of tumor-bearing mice in comparison to similar nonlevanized animals. For example, in the experiment described in Chart 1, 50% mortality occurred on the 38th day after tumor inoculation, and on the 28th, 27th, and 24th days in animals treated with 5, 10, and 25 mg levan, respectively.

Microscopic Findings. Histological study of the tumors at the site of injection showed that levan administration was associated with a decrease in cellular pleomorphism and a decrease in the number of mitoses. Both these phenomena were related to the dose of levan. With increasing levan concentrations, evidence of progressive damage to tumor cells was noted. Some cells were shrunken and hyperchromatic, and the presence of nuclear debris (Fig. 2) indicated necrosis. Invasion of neighboring structures by tumor cells was less prominent in levanized than in control animals. In levanized mice, the tumor was mostly well circumscribed.

Also in the liver, differences in pleomorphism, mitoses, and cell damage were similar to those observed in the other metastatic tumors. In addition, the distribution of tumor cells was markedly different in accordance with the dose of levan. In untreated mice, tumor cells were concentrated mainly in and around the portal tracts. With decreasing doses of levan, more and more cells were found in the

Table 2

Weight of primary and metastatic tumors and spleens in AKR mice treated by increasing concentrations of levan

Mice in groups of 3 were sacrificed on the 34th day after inoculation of 10^5 tumor cells.

Amount of levan injected/ day (mg)	Primary tumor		Mesenteric lymph nodes		Spleen	
	Mean wt (mg) ± S.E.	p	Mean wt (mg) ± S.E.	p	Mean wt (mg) ± S.E.	p
	501 ± 256		481 ± 152		491 ± 182	>0.05
5	177 ± 126	>0.05	235 ± 112	>0.05	365 ± 30	>0.05
10	84 ± 53	<0.05	170 ± 22	<0.05	384 ± 46	>0.05
25		<0.01	204 ± 96	<0.05	367 ± 178	>0.05

hepatic lobules, mainly in dilated sinusoids, with compression and destruction of hepatocytes. Also, in control animals receiving levan but not inoculated with tumor cells, the hepatic sinusoids were dilated.

DISCUSSION

In the present experiments, levan was found to inhibit the main manifestations of neoplastic development in the mouse, *i.e.*, the growth of the primary tumor, its invasiveness into surrounding tissues, and its metastatic spread. The effects were obviously dose dependent.

The inhibition of tumor development could have been due to a direct effect of levan on the tumor cells, to an effect on the host tissues, or to modulation of the immune response of the host.

A direct effect of levan on the tumor cells is suggested by the decreased mitotic rate and pleomorphism and the evidence of cell destruction in levanized mice. Levan might also effect the host response to the tumor, possibly by preventing the growth of new capillaries into the tumor. It has been shown that levan inhibits vascularization of cutaneous wounds (21).

The administration of levan was found to inhibit the invasiveness of the tumors. In levanized mice, tumors appeared more encapsulated and usually did not infiltrate the surrounding tissues. This effect might have been caused by the decreased rate of tumor growth. It has been proposed that surface polysaccharides affect the pattern of adhesion in malignant cells (17, 18). Cell adhesion is a major factor in cancer (1). The presence of high-molecular levan between cells might alter the surface of the tumor cells and subsequently alter adhesiveness and invasiveness. Levan was shown to inhibit metastatic spread of the tumors. This inhibitory effect may also be explained by the decreased rate of growth and invasiveness shown in our experiments. In addition, the inhibition of metastatic spread may be related to coating by levan of the endothelial linings of blood vessels and changes in their permeability (3, 7).

The different distribution of tumor cells in the livers of levan-treated animals in comparison to their distribution in nonlevanized mice resembles the difference between myelogenous and lymphatic leukemias. In levanized mice the hepatic sinusoids were dilated and packed with tumor cells. Dilatation of hepatic sinusoids was also present in levanized mice that had not been inoculated with tumor cells. It is not clear whether mechanical or other factors are responsible for these differences.

The increased mortality rate in tumor-bearing levanized animals has not been explained. The effect is not due to the administration of levan *per se*, as no such mortality was observed in levan-treated animals not inoculated by tumor cells. The animals might have been affected by the massive tumor cell destruction observed in the levanized animals, possibly by blockade of the reticuloendothelial system, or by some other mechanism. In unpublished experiments we (Y. Sinai, J. Leibovici, and M. Wolman) found that the lethal effect of levan on tumor-bearing animals can be obviated by changes in the route and schedule of levan administration.

The various aspects of inhibition of tumor development discussed in the preceding paragraphs may be related to a modification of the immunological response of the host effected by levan. Levan and other polysaccharides were shown, in fact, to be immunological modulators acting as B-cell mitogens and having no effect on T lymphocytes (2, 5, 6, 8, 9, 11, 13–16). This selective effect of levan on the 2 lymphocyte populations might enhance the immunological defense of the host against tumor cells.

The effect of levan on the immunological response of the host to tumor cells might also result from the prevention of shedding of membrane antigens from tumor cells. Shedding of antigens (10) and the subsequent blocking of the immune response to tumors by antigen-antibody complexes has been reported (4). The mechanism by which levan might interfere with shedding of antigens could be related to coating of tumor cell surfaces by the polysaccharide.

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Fig. 1. Comparison between mesenteric lymph node metastases in animals treated with different doses of levan. *A*, no levan; marked pleomorphism of cells and numerous mitoses; *B*, 5 mg levan daily; less marked pleomorphism; *C*, 10 mg levan daily; little pleomorphism, few mitoses and presence of some cellular debris; *D*, 25 mg levan daily; no mitoses, pyknotic nuclei and presence of nuclear dust, little pleomorphism. H & E, × 360.

Fig. 2. Spread of tumor in liver. *A*, no levan. Tumor cells are mainly concentrated around portal vein with little penetration into lobule. *B*, 25 mg levan daily. Tumor cells have spread in parenchyma, lying in sinusoids, with severe compression and destruction of hepatic cells. H & E, × 360.