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Effect of Native Levan on Homograft Rejection in Mice (38805)

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Delay of graft rejection is of great clinical importance and is usually achieved by immunosuppressors. These include radiation, chemical agents (1), and biological agents such as antilymphocyte serum (ALS) (2), interferon (3), and enzymes (4). There is an obvious need for alternative methods for inhibiting graft rejection, as the presently used procedures are often accompanied by untoward and dangerous reactions. Radiation and chemical immunosuppression are sometimes complicated by opportunistic viral and mycotic infections and also by tumors (3). ALS may be deleterious as it may contain antibodies against organs and because of its stimulatory effect on the immune response (2). Alternative graft-rejection suppressors might be of clinical importance for temporary or permanent replacement therapy when the use of presently available agents has to be discontinued.

High-molecular-weight levan has been shown to inhibit the inflammatory response by preventing transport of proteins including immunoglobulins (5, 6) and diapedesis of cells across the endothelium (7). The decreased permeability of blood vessels caused by levan might inhibit graft rejection either by preventing the afferent (sensitizing) or the efferent (cytolytic) paths, or both parts of the rejection process.

Materials and Methods. Male, 10-wk-old mice were obtained from the Weizmann Institute, Rehovoth. Balb/c and C57BL mice were used as donors and recipients. Levan was purchased from the Technical Unit Department of Biological Chemistry of the Hebrew University in Jerusalem. The polysaccharide was prepared according to Hestrin, Shilo, and Feingold (8). The molecular weight was approximately 2×10^7. Five percent levan solutions were prepared in saline according to Shilo, Wolman, and Wolman (7). Injection of levan was performed according to the following schedule: 15 mg were injected ip 8, 6, and 4 days before transplantation (a procedure which was found to reduce animal mortality during subsequent levanization) followed by daily ip injections of 15 or 30 mg beginning on the day before grafting. Skin transplantation was performed according to Billingham and Silvers (9) with slight modifications. After shaving, residual hair was removed by BaS. Skin was then cleansed with ethanol and ether. Grafts were obtained with a punch 5 mm in outer diameter. The transplants were dressed with Liquidoplast (Dr. Hammer and Co. GMBH, 2 Hamburg 13), covered by Micropore surgical tape (Blenderm 3M Company, St. Paul, MN) with a window of cellophane to permit daily inspection. The fate of the grafts was also followed by histologic examination in selected animals.

Each animal was grafted with isologous and homologous skin. The isografts served as controls for the technique and were accepted in 90% of nonlevanized and levanized animals.

Results. An increase in graft survival in levan-treated mice was shown in Balb/c and C57BL recipient mice compared to nonlevanized animals (Figs. 1 and 2). The maximum graft survival achieved was 23 days after transplantation in levan-treated as compared to 11 days in the control mice.

The MST value (mean survival time in days) increased in animals treated with levan (Table I): 12.2 vs 8.6 in Balb/c recipients ($P < 0.01$) and 13.8 vs 8.2 in C57BL recipients ($0.1 > P > 0.05$).

The difference in rejection time in levanized vs untreated animals was most obvious

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on Days 8–10 after transplantation. In Balb/c mice rejection of homografts occurred in 59% of nonlevanized mice. In C57BL mice the grafts were rejected at that period in 75% of control animals and in none of the levanized ones.

The macroscopic evaluation of graft rejection was verified in some animals by histological study.

Discussion. High-molecular-weight levan has been shown to have a significant inhibitory effect on graft rejection. In preliminary work, dextran having a molecular weight of 2×10^6 did not show this effect. The inhibitory effect of levan on graft rejection was significant with Balb/c mice as recipients. With C57BL recipients, an inhibitory effect of levan was obtained, but because of the small number of animals the results were on the threshold of significance.

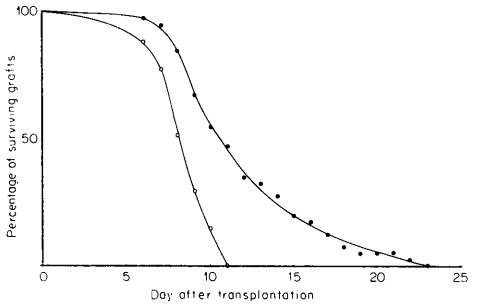

FIG. 1. Effect of daily levan administration on survival of skin grafts in Balb/c recipient mice. Empty circles, nonlevanized mice (27 animals). Black circles, levanized mice (40 animals).

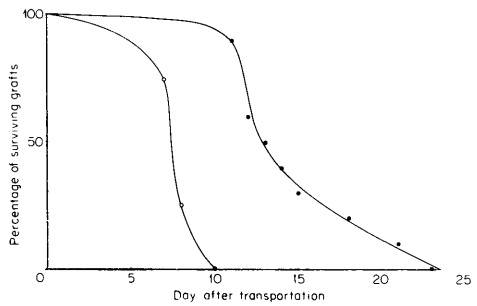

FIG. 2. Effect of levan on survival of skin grafts in C 57/BL recipient mice. Empty circles, nonlevanized mice (4 animals). Black circles, levanized mice (10 animals).

The high variability in the rejection time in levan-treated compared to control mice might be related to the ip route of levan administration.

The observation that levan with its simple molecular structure delays graft rejection might be of practical significance as it might be less deleterious to the host than most antimitotic agents. In the concentrations used in this study levan is nonimmunogenic (10).

The precise mechanisms of graft rejection delay by levan has not been clarified in the present experiments. Levan may act on the afferent and/or the efferent arc of the immune response. The polysaccharide may either prevent antigens of the graft from reaching and sensitizing lymphocytes and macrophages, or disturb the passage of sensitized cells into the graft. Inhibition of antigen processing may also be involved, as levan has been shown to affect the reticuloendothelial system (11). A cytotoxic effect of levan on one or more of the categories of cells involved in graft rejection may also be considered. Studies to test these possibilities are now in course.

Summary. The effect of high-molecular-weight levan on skin graft rejection was studied. Daily ip administration of 15–30 mg levan was shown to delay rejection in Balb/c and C57BL recipient mice. An increase in MST value by 3.6 days was obtained in Balb/c mice and of 5.6 days for C57BL.

TABLE I. EFFECT OF LEVAN TREATMENT ON SURVIVAL OF SKIN HOMOGRAFTS.

	Donor Strain	Recipient strain	Number of mice	MST	*P*
Control	C57 BL	Balb/c	27	8.6 ± 1.5	<0.01
Levan treated	C57 BL	Balb/c	40	12.2 ± 4.1	
Control	Balb/c	C57 BL	4	8.2 ± 1.2	$0.1 > P > 0.05$
Levan treated	Balb/c	C57 BL	10	13.8 ± 5.2	

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