Suppression of humoral immune response in mice by administration of high molecular levan

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Summary. High molecular levan, a polyfructoside, has a dose-dependent inhibitory effect on the primary immune response to sheep red cells (SE) in Balb/c mice, when given as from 1-2 days prior to the antigen injection. A slight stimulation of the immune response was observed when levan was given shortly before or 1 day after the antigen.

Administration of native high molecular levan (LE) has been shown in our laboratory to be active in delaying graft rejection[1], inhibiting experimental allergic encephalomyelitis[2] and to exhibit an antitumor effect[3,4]. Immunological reactions are known to be involved in all these pathological processes. It was therefore of interest to study the effect of levan on immune responses in animals, in conditions similar to those used by us for treatment of the above disorders. Levan has been found by others to affect the humoral immune response as a T-independent B cell mitogen[5]. In the present communication we have studied the effect of levan administration in different doses and schedules on the primary immunological response to SE (sheep erythrocytes) by determination of plaque-forming cells (PFC) in spleens and by titrating the hemagglutinins (HA).

Materials and methods. Balb/c male mice were obtained from the Animal Breeding Center of the Weizmann Institute of Science, Rehovot, Israel. Levan prepared according to Hestrin et al.[6] was purchased from the Technical Unit, Department of Biological Chemistry of the Hebrew University of Jerusalem. The average mol. wt was approximately 2×10^7 daltons; 5% solutions were prepared in saline according to Shilo et al.[7]. Various doses of levan were daily injected i.p., beginning on different days in relation to the antigen injection.

Mice were immunized by an i.p. injection of 0.1 ml of a 10% suspension of PBS-washed packed SE. Hemolytic plaque assay was performed according to Jerne et al.[8], except that agarose A-37 (Industrie Biologique Francaise S.A., Gennevilliers, Seine, France) was substituted for agar. Fresh guinea-pig serum adsorbed with SE was used as a source of complement. 2fold dilutions of sera were assayed for anti SE antibodies by direct haemagglutination.

Results. Figure 1 shows the effect of different doses of LE injected beginning at 24 h before SE on the number of PFC. An inhibitory effect (32%) was evident already with a 10 mg/mouse daily dose. The inhibitory effect was dose-dependent until 15 mg. Higher doses did not increase this effect, the maximum inhibition being of 84%.

Figure 2 presents the effect of different doses of LE on the HA titer at various times after antigen administration. A definite reduction in the titer of hemagglutinins was obtained with each of the concentrations used on days 4,7 and 15 after immunization. No significant difference in the inhibition of HA was observed with daily doses of levan ranging between 5 and 30 mg.

Figure 3 shows the effect of the time of LE administration in relation to the antigen injection. In animals pretreated with LE beginning 1 or 2 days before immunization, a PFC reduction of 72% and 61% respectively was obtained. LE injected 30 min before or 24 h after the antigen did not diminish the immune response to SE. On the contrary, a slight stimulation (22-26%) was observed.

Discussion. LE reduced significantly the immune reaction to SE as judged by PFC and hemagglutinin appearance, when given 1 or 2 days before immunization. The inhibition was dose-dependent beginning with 10 mg levan per mouse. Maximum inhibition was achieved at a 500-mg

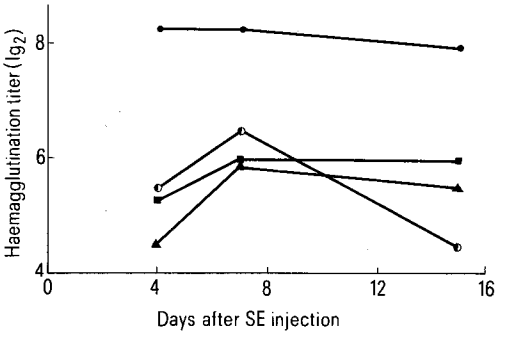

Fig. 2. Effect of levan on hemagglutination titer. Groups of 3 mice were injected i.p. with different daily doses of levan beginning 24 h and continued until the animals were sacrificed. Hemagglutination titer was assayed in the sera of animals on days 4, 7 and 15 after immunization, by direct agglutination test in microplates. ●, non-treated, levan treated with 5 mg ■, 15 mg ▲, and 30 mg ◐.

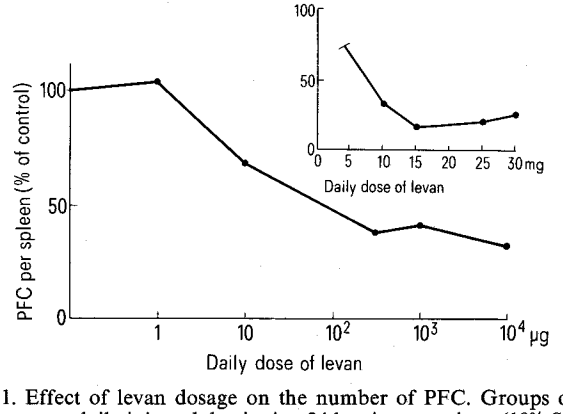

Fig. 1. Effect of levan dosage on the number of PFC. Groups of 5 mice were daily injected, beginning 24 h prior to antigen (10% SE in PBS) injection and continued until the 4th day after, when the mice were sacrificed and PFC test performed on the spleens.

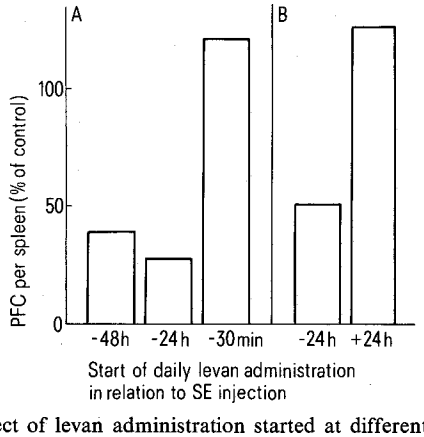

Fig. 3. Effect of levan administration started at different times in relation to antigen injection on PFC counts. Same conditions as in figures 1 and 2. *A* 1 mg levan; *B* 15 mg levan.

dose. No significant increase in the inhibitory effect was obtained at higher doses. No inhibition in PFC number and HA titre was observed in mice which were treated with LE beginning shortly before or after immunization.

Similar results were obtained with other B cell mitogens by Diamantstein et al.[9]. These authors suggest that stimulation of antigen-sensitive cells by B-cell mitogens a few days before immunization may lead to formation of a cell population which has lost its ability to react with antigens.

We propose 2 other possibilities for explaining the different effects of levan administered 24–48 h before antigenic stimulation and the effect of levan administration just before or after the antigen injection. a) LE injected before the antigen may diminish the antigen's availability to the immune system by decreasing the permeability of blood vessels[10]. b) LE injected before the antigen may block the RES (reticuloendothelial system), thereby preventing antigen processing. LE administered around the time of antigenic stimulation cannot block in time the already started antigen processing. Levan is known to have a variety of effects on macrophages[4,11,12], and these cells were shown to be responsible for some differences in immune response between high and low responder strains of mice[13].

The use of LE in the treatment of various pathological processes requires in some cases stimulatory, in others inhibitory effects on different immunological reactions. In the graft rejection process, the aim is to preserve the grafted tissue, while in tumor therapy the aim is to destroy the neoplastic cells. During the development of tumors, some immunologic reactions enhance, while others inhibit growth of the neoplasm. Studies of our group demonstrated that levan treatment inhibited tumor growth only when the daily injections were started within a few days after tumor-cell inoculation. Treatment with LE begun even 1 day before the tumor inoculation had an enhancing effect[14]. Similar effects were also noted with other immunomodulatory agents[15]. Whether the dependence of antitumoral activity on the time at which LE treatment is started, and the effect on antibody production of the period at which LE is given, are due to the same mechanism, is not clear.

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Immunoadjuvant activity of synthetic N-acetyl muramyl dipeptide

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Summary. The administration of phosphatidyl choline/cholesterol liposomes with entrapped N-acetyl muramyl dipeptide induced in guinea-pigs the development of delayed hypersensitivity to ovalbumin.

Several decades ago Freund and McDermott[1] discovered the immunoadjuvant property of mycobacteria with water-in-oil emulsion. The search for adjuvant active component of mycobacteria led first to the isolation of peptidoglycolipid fraction called Wax D[2,3]. Soon it became clear that the lipid is not essential for the observed activity, and water-soluble preparation of glycopeptide replaced mycobacteria in the Freund complete adjuvant for stimulation of delayed hypersensitivity[4–6]. Several groups independently then discovered that the minimal structure which is required for immunoadjuvant activity is N-acetyl muramyl L-alanyl-D-isoglutamine[7,8].

Chedid et al.[9] recently reported that some synthetic glycopeptides are even able to increase the humoral immune response when given in aqueous media instead of the usual water-in-oil emulsion. This, however, has not been observed so far for the development of cell mediated immunity (delayed hypersensitivity) where water-in-oil emulsion is needed to note the effect. The mineral oils used in Freund's adjuvants are not biodegradable, and they can be thus used only in the experiments on animals. Liposomes, phospholipid vesicles consisting of one or more lipid layers, appear to be promising as carriers of antigens to immunocompetent cells. Several recent studies have reported that liposomes increase the antibody response to a variety of protein antigens[10,11]. The main objective of the present study was to elucidate whether the liposomes can substitute mineral oils

Immunoadjuvant activity of N-acetyl-muramyl dipeptide

Compound tested	Dose	Delayed hypersensitivity
Ac-Mur-L-Ala-D-Glu-NH_2	200 µg	7.94
Phosphatidyl choline-cholesterol (8:2)		8.86
Pch-Ch + Ac-Mur-L-Ala-D-Glu-NH_2	200 µg	12.75*
FIA + Ac-Mur-L-Ala-D-Glu-NH_2	200 µg	13.82*
Freund's complete adjuvant (FCA)	1000 µg	13.58*
Freund's incomplete adjuvant (FIA)		7.34

Values represent the mean values for group of 8–10 guinea-pigs.
* Statistically significant results; $p < 0.05$.