Immunosuppressive Activity of Concanavalin A

Abstract. *Two strains of mice (C-57 Bl and DBA) were given skin allografts from Swiss ICR/HA mice and were treated with varying doses of concanavalin A; the dosage ranged from only two injections of 25 micrograms during the experiment to daily doses of 1000 micrograms intraperitoneally. Immunosuppression was present at all dosages, the most prolonged and consistent effect being observed at 300 micrograms per day. Grafts survived for 14 to 21 days at this dosage compared to 4 to 9 days for controls.*

The phytohemagglutinin from the red kidney bean (*Phaseolus vulgaris*) is capable of inducing lymphocyte transformation (*1*). There is evidence that it participates in the immune process leading either to depression (*2*) or to potentiation or no response (*3*) of both primary and secondary antibody production. Since both the experimental conditions and the source and type of the phytohemagglutinin varied, no definite conclusions could be drawn. Results regarding the effect of phytohemagglutinin on skin allograft survival are similarly conflicting (*4*).

We have been concerned with elucidation of structure of polysaccharides using the phytohemagglutinin, concanavalian A (con A), derived from the jack bean (*Canavalia ensiformis*). Concanavalin A is obtained from jack bean meal as a homogeneous protein (*5*) that is free of sulfur, has a molecular weight of 68,000, and is a potent hemagglutinin. It interacts with serum glycoproteins (*6*), carbohydrates, and nucleic acids (*7*). In addition, con A induces lymphocyte transformation (*8*). We investigated the ability of this fairly well-defined protein to act as an immunosuppressive agent because labeling and degradative procedures would be possible, and a mechanism of action could be sought if con A were found to prolong allograft survival.

Concanavalin A was prepared from jack bean meal (*9*) by a slight modification of the method of Agrawal and Goldstein (*5*). The product was ultracentrifugally homogeneous (S_{20} was 6.05 in 0.01*M* phosphate-buffered saline, *p*H 7.2), contained 0.022 percent Mn and 0.02 percent P, and was highly active as a hemagglutinin and in quantitative precipitin reactions with dextran B-1355S (*10*) and polysaccharides from *Histoplasma capsulatum*. Full-thickness allograft recipients were C-57 Bl/6J and DBA/2J female mice, and Swiss ICR/HA mice were used as donors (*11*). Each group of mice included two animals that received an autograft as well as an allograft. Concanavalin A was given by intraperitoneal injection of 0.1 ml of solutions containing 10, 6, 3, 1, and 0.25 mg/ml (in 0.01*M* phosphate-buffered saline, *p*H 7.2). All con A preparations were filtered (Millipore, 5 μ) and stored frozen without preservative. Mice were injected with the quantities noted at 24 hours before and 24 hours after grafting. Groups noted as "daily" were injected with con A daily until termination of the experiment.

Table 1. Effect of concanavalin A on allograft survival. Treatment was by injection (in two categories); A, daily until the end of the experiment; B, two injections only, 24 hours before and 24 hours after grafting.

Treatment		Mice (No.)	Days of graft survival		
Dosage (μg)	Type		4–10	11–15	16–22
None		25	25		
1000	A	4		4	
1000	B	4		3	1
600	A	8	1	3	4
600	B	3		1	2
300	A	14		5	9
300	B	5		5	
100	A	6		6	
100	B	6	3	2	1
25	A	7	2	3	2
25	B	6	1	4	1

The results indicate an immunosuppressive action of con A at all dosages used (Table 1). Rejection of the grafts was slow and progressive rather than prompt. Occasionally the rejection began as a central superficial hemorrhagic area that spread radially to the viable periphery of the allograft. Only at the lowest dose of con A did rejection ever resemble that in the controls (prominent peripheral graft reaction and eschar formation). Grafts were considered rejected if 50 percent of the skin was hemorrhagic or sloughed, even if the remainder was intact. The animals appeared to be in good health throughout the experiments despite large cumulative doses of con A.

Postmortem examination of tissue indicated only a marked peritoneal inflammatory reaction in animals at a dose of 300 μg/day. However, with 1000 μg/day, large white peritoneal masses were found, which we hypothesize may be due to precipitation of the injected con A with available carbohydrate and glycoprotein. This was the only group with high animal mortality (50 percent). All simultaneous autografts were completely accepted well beyond the duration of the experiment. After the therapy was stopped, no precipitating antibody was present in serums from several mice treated with various dosages. These determinations were made by microimmunodiffusion and treatment of plates with 0.1*M* methyl-α-D-mannopyranoside in 0.01*M* phosphate-buffered saline, *p*H 7.2, to eliminate nonspecific bands.

Our results suggest that con A is effective as an immunosuppressive agent at low dosages and that, in short-term experiments, it is well tolerated. The mechanism of this action is unknown, although con A may act differently from the phytohemagglutinin from *Phaseolus vulgaris* since con A is a sulfur-free protein.

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References and Notes

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11. The C-57 Bl/6J and DBA/2J female mice were obtained from Jackson Laboratory, Bar Harbor, Maine. Swiss ICR/HA mice were from a colony maintained at the Mayo Clinic.
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