Inhibition of Delayed Hypersensitivity to Tuberculin by Concanavalin A* (33964)

M. A. Leon and H. J. Schwartz
(O. D. Ratnoff)

Department of Pathology Research, St. Luke's Hospital, Cleveland, Ohio 44104; and Department of Medicine, Case Western Reserve University, Cleveland, Ohio 44106

Concanavalin A, a lectin obtained from the jack bean, has two sites per molecule capable of binding sugars with the D-arabinopyranoside configuration at C-3, C-4, and C-6 (1). As a consequence of this specificity, concanavalin A combines with a variety of glycoproteins (2–4) and polysaccharides (5). Cells such as erythrocytes and lymphocytes, which contain such reactive macromolecules, bind concanavalin A as shown by studies with ^{125}I labeled concanavalin A (6, 7). In the case of lymphocytes, the binding initiates the series of reactions leading to blast transformation (7).

The present paper reports that the intraperitoneal injection of concanavalin A markedly suppresses the delayed hypersensitivity responses of guinea pigs sensitized to mycobacteria.

Materials and Methods. After a 10-day acclimitization period, random bred, female, albino guinea pigs (Starrett Farms, Akron, Ohio), weighing 400–500 g, were immunized with complete Freund's adjuvant (Difco, containing Arlacel A, 1.5 ml; Bayol F, 8.5 ml; and *M. butyricum* (killed and dried), 5 mg) by intradermal injection of 0.1 ml into each posterior foot pad and subcutaneous injection of 1.0 ml into one site on the back. The animals were fed Rockland guinea pig ration and water *ad libitum*, with daily supplements of fresh lettuce. Three weeks later, the animals were shaved with a Oster model no. A-2 animal clipper, depilated with Nair (Carter Products) and skin tested with 0.1 ml of old tuberculin diluted 1:200 in saline. At the same time, one group of animals received 2 ml of sterile saline by intraperitoneal injection. Two experimental groups were given either 10 mg or 1 mg of concanavalin A in 2 ml of saline intraperitoneally. Sites were measured at 24 hr for induration and erythema.

Concanavalin A was prepared by minor modification (4) of the method of Agrawal and Goldstein (5). Concanavalin A labeled with ^{125}I was prepared by the method of Helmkamp *et al.* (8). Egg albumen (5 × recrystallized) was purchased from Pentex, Inc. Old tuberculin was purchased from Parke, Davis.

Results. The data (Table I) demonstrate that intraperitoneal administration of either 10 mg or 1 mg of concanavalin A to tuberculin-sensitive guinea pigs, at the time of skin test challenge, significantly suppresses the delayed reaction. One animal in the group receiving 10 mg died overnight, but there were no obvious signs of toxicity in the survivors. Preliminary data showed that intraperitoneal injection of concanavalin A 1 hr after intradermal challenge with 0.1 ml of 1:200 old tuberculin also suppressed delayed reactivity.

The glycoprotein, egg albumen, forms soluble complexes with concanavalin A, thereby blocking binding sites on the concanavalin A which could react *in vivo* with guinea pig glycoproteins either on cell surfaces or in body fluids. Accordingly, in a second experiment concanavalin A was reacted for 30 min at 37° with an excess of egg albumen and the suppressive effect of this mixture was compared with similarly treated mixtures of concanavalin A and saline, and egg albumen and saline. The data in Table II indicates that prior complexing of concanavalin A with egg albumen prevents the concanavalin A from acting as a suppressant of the delayed response.

Distribution of ^{125}I labeled concanavalin A 1 hr after injection of 5 mg intraperitoneally

* Supported in part by USPHS Grant No. AI03104, the Cuyahoga County Unit of the American Cancer Society, and USPHS Grant No. AM01005.

TABLE I. Inhibition of Tuberculin Hypersensitivity by Intraperitoneal Injection of Concanavalin A.

Treatment		Delayed reactions to skin tests with OT[a]
Con A 10 mg	er	0,0,0,0,0,0,0,0,0,9,8
	ind	0,0,0,0,0,0,0,0,0,0,0
1 mg	er	0,0,0,0,0,0,10,3,3,10,6,10
	ind	0,0,0,0,0,0,0,0,0,0,0
Saline	er	9,9,11,5,13,12,11,8,5
	ind	+,++,++,+,++,0,++, ++,0

[a] Degree of induration was judged by palpation. All reactions are shown as measured. Hence, there were no minimum criteria needed for a positive test. Abbrev.: er = erythema; ind = induration; numerals = mm of erythema; + = slight induration; and ++ = induration.

showed that the major portion of the radioactivity remained in the abdomen. Approximately 0.5% was found in the blood and approximately 0.06% in a skin site.

Discussion. The data presented here demonstrate that concanavalin A suppresses established delayed hypersensitivity to tuberculin. Jasin and Ziff reported that another plant lectin, phytohemagglutinin-P, had no effect on tuberculin sensitivity in rats (9). Our experiments differ in that they deal with established delayed sensitivity rather than with the induction of such immunity. Thus, their administration of phytohemagglutinin-P at the time of immunization rather than at the time of skin testing renders comparison difficult. There is a suggestion, however, that concanavalin A can also operate at the stage of induction of cellular immunity. Administration of concanavalin A both 24 hr before and after skin grafting in mice results in prolonged survival (10).

TABLE II. Inhibition of Concanavalin A Effect by Prior Reaction with Egg Albumen.

Treatment		Delayed reactions to skin tests with OT
Con A–EA[a]	er	8,12,9,7,7
	ind	+,++,++,+,0
EA, 40 mg	er	10,9,7,6,10,9
	ind	++,0,0,+,++,++
Con A, 5 mg	er	3,3,0,2
	ind	0,0,0,0

[a] Concanavalin A (5 mg) incubated with egg albumen (40 mg) for 30 min at 37° in total volume of 4.2 ml saline, injected intraperitoneally at time of challenge; controls incubated with saline solution.

Since concanavalin A complexes with glycoproteins and possibly glycolipids at the surface of a variety of cells (6, 7), and also complexes with glycoproteins in body fluids (3–5), the site of its action in suppressing tuberculin reactivity cannot at present be specified. However, the finding that the suppressive action of concanavalin A was abolished by prior complexing with egg albumen suggests that the carbohydrate combining sites of concanavalin A are responsible for its action. Concanavalin A can be dissociated readily from complexes with glycoproteins and polysaccharides, using the mild reagents methyl alpha-D-glucoside and methyl alpha-D-mannoside. This approach provides a wealth of experimental possibilities for studies with passive transfer systems to elucidate the site of the suppressive action.

Summary. Intraperitoneal inoculation of concanavalin A into tuberculin-sensitive guinea pigs at the time of skin testing markedly suppressed the delayed reaction. This effect was abolished when concanavalin A was complexed with egg albumen prior to intraperitoneal inoculation.

1. So, L. L. and Goldstein, I. J., Biochim. Biophys. Acta **165**, 398 (1968).

2. Nakamura, S., Tanaka, K., and Murakawa, S., Nature **188**, 144 (1960).

3. Harris, H. and Robson, E. B., Vox Sanguinis **8**, 348 (1963).

4. Leon, M. A., Science **158**, 1325 (1967).

5. Agrawal, B. B. L. and Goldstein, I. J., Biochem. J. **96**, 23c (1965).

6. Yokohari, R., Jocius, I., and Leon, M. A., Federation Proc. **27**, 314 (1968).

7. Leon, M. A. and Powell, A. E., Res, J. Reticuloendothelial Soc. **5**, 35 (1968).

8. Helmkamp, R. W., Goodland, R. L., Bale, W. F., Spar, I. L., and Mutschler, L. E., Cancer Rs. **20**, 1495 (1960).

9. Jasin, H. E. and Ziff, M. D., Immunology **14**, 735 (1968).

10. Markowitz, H., Person, D. A., Gitnick, G. L., and Ritts, R. E., Jr., Science **163**, 476 (1968).

Received Feb. 19, 1969. P.S.E.B.M., 1969, Vol. 131.