Concanavalin A-Induced Sensitization of Mice to Histamine (34154)

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In recent years a group of plant proteins called phytohemagglutinins have been isolated and found to have interesting properties (1). For example, concanavalin A, the jack bean phytohemagglutinin, reacts with polysaccharides to form insoluble complexes with a reaction profile which parallels the classical antigen–antibody precipitin curve (2). The antigen–antibody character of concanavalin A–carbohydrate mixtures has been demonstrated by a variety of immunochemical techniques, including hapten inhibition, gel diffusion, and immunoelectrophoresis (2, 3). Bacterial endotoxins which contain the proper carbohydrate determinant groups precipitate with concanavalin A (4), and a variety of bacteria are agglutinated by concanavalin A.

In view of these interactions of this phytohemagglutinin with intact bacteria and bacterial products, we were interested in determining whether this phytohemagglutinin would modify the histamine sensitivity induced by *Bordetella pertussis*. This species of bacteria renders certain strains of mice, normally resistant to histamine, sensitive to low doses of the amine. Although the data show that concanavalin A does not alter the effects of pertussis, the phytohemagglutinin alone was found to be a potent sensitizing agent. This is the first report of the ability of a purified protein to elicit histamine sensitization in mice.

Materials and Methods. Female mice, strains CFW and CFI, averaging 20 g, were obtained from Carworth Farms. All animals were adapted to the laboratory environment for a minimum of 1 week prior to use, and supplied food and water *ad libitum*. Following the initial injection, they were housed five animals per cage.

Concanavalin A was isolated from defatted jack bean meal (Sigma Chemical Co., St. Louis, Mo.) according to the method of Agrawal and Goldstein (5). Solutions of concanavalin A were prepared in sterile, nonpyrogenic, physiological saline solution, filtered through a 0.2-μ filter, and absorbance at 280 mμ was used to calculate the final concentration (6). Dilutions were made in saline solution.

Suspensions of *B. pertussis* were supplied through the courtesy of Eli Lilly and Co. (Indianapolis, Ind.). Pertussis-treated controls were inoculated subcutaneously with 0.5 ml of a 1:2 concentration diluted in saline. Animals receiving the combined drugs were injected subcutaneously with 0.5 ml of concanavalin A diluted in the pertussis suspension to yield a 1:2 concentration of pertussis and 0.5 mg of concanavalin A. For the experiment testing concanavalin A with glucose, 0.5 mg of D-glucose was dissolved in the concanavalin A solution and injected subcutaneously. All animals in these groups, together with saline controls, were challenged with histamine 5 days after the initial injection.

The animals tested with concanavalin A alone were injected either intraperitoneally or subcutaneously with a volume of 0.5 ml. The dose, injection route, and time intervening between the sensitizing injection and histamine challenge is specified for each experiment.

Histamine diphosphate (Nutritional Bioch. Corp., Cleveland, Ohio), was dissolved and diluted in saline solution immediately prior to use, and administered intraperitoneally in a volume of 0.2 ml. The challenge dose, 0.62 mg, represents amine base. Deaths were recorded for a 60-min period following challenge.

TABLE I. Histamine Sensitivity of CFW and CFI Mice 5 Days after Administration of Concanavalin A and Pertussis.

Strain	Sensitizing drug	Dose	Histamine lethality[a]
CFW	Saline (control)	0.5 ml	1/25
	Pertussis	1:2	22/25
	Pertussis + concanavalin A	1:2 0.5 mg	20/25
	Concanavalin A	1.0 mg	10/20
	Concanavalin A	0.5 mg	15/30
	Concanavalin A	0.25 mg	12/25
	Concanavalin A	0.12 mg	8/25
	Concanavalin A	0.06 mg	4/25
	Concanavalin A	0.03 mg	4/25
	Concanavalin A + D-glucose	0.5 mg 0.5 mg	13/25
CFI	Saline	0.5 ml	0/10
	Pertussis	1:2	0/10
	Pertussis + concanavalin A	1:2 0.5 mg	0/10
	Concanavalin A	0.5 mg	0/10
	Concanavalin A + D-glucose	0.5 mg 0.5 mg	0/10

[a] Deaths/total animals injected.

Results. The data in Table I show that concanavalin A did not alter significantly the pertussis-induced sensitization of CFW mice, but the phytohemagglutinin alone rendered these mice sensitive to histamine. The response to the challenge dose of histamine is a function of the dose of concanavalin A, ranging from an LD_{16} at 0.03 mg to an LD_{50} at 0.5 mg. The death rate was not increased by concentrations greater than 0.5 mg.

Table II shows the response of strain CFW as a function of the time intervening between concanavalin A administration and histamine challenge. It is apparent that sensitization following subcutaneous administration increases as a function of time, peaks at 4 days, is maintained at the peak through the fifth day, and decreases after 5 days. However, the intraperitoneal administration of concanavalin A followed by immediate challenge produced lethality equivalent to that obtained 3 days after subcutaneous administration.

None of the mice injected with concanavalin A died as a result of the phytohemagglutinin, nor did any manifest behavioral symptoms of toxicity. However, the subcutaneous administration of concanavalin A in CFW and CFI mice did induce a marked edema which radiated approximately 1 cm from the injection site. The size of this edematous mass varied in proportion to the dose of concanavalin A, was maximal at 24 hr, and subsided gradually until it was no longer apparent at the end of 6 days. Since both strains showed identical local effects, we do not consider this local reaction to be related directly to histamine sensitization.

Discussion. The unexpected ability of concanavalin A to induce sensitization to histamine prevented an assessment of interactions between the phytohemagglutinin and the sensitizing factors of *B. pertussis*. Since the death rate from the combined drugs was the same as that induced by pertussis alone, but higher than the maximum rate achieved with concanavalin A, the phytohemagglutinin obviously did not decrease the potency of pertussis. This dose of pertussis resulted in a death rate too close to 100% to permit an assessment of synergism with concanavalin A. Current studies include the titration of pertussis to a death rate low enough to test for combined effects.

TABLE II. Sensitization of CFW Mice by Concanavalin A as a Function of Time.

Days[a]	Injection route[b]	Histamine lethality
0	iv	0/10
0	ip	4/25
0	sc	1/25
1	sc	0/25
2	sc	1/25
3	sc	5/25
4	sc	13/25
5	sc	15/30
6	sc	7/25

[a] Days between injection of 0.5 mg concanavalin A and challenge with histamine. "0" days indicate challenge immediately after administration of concanavalin A.

[b] Abbrev.: iv = intravenous; ip = intraperitoneal; sc = subcutaneous.

In terms of current theories regarding induced histamine sensitization in mice, there are at least two possible explanations for these results. One concerns beta-adrenergic blockade which, by altering pathways mediated by catecholamines, prevents the operation of the regulatory mechanism which normally protects these animals from the effects of histamine on susceptible tissues. This is the mechanism proposed for pertussis sensitization (7). There are certain noteworthy similarities between pertussis and concanavalin A: the subcutaneous injection of both requires a period of time to elicit maximum sensitization, the peak response for both occurs at approximately the same time, and both sensitize CFW but not CFI animals.

Since there is evidence for an antigen–antibody-like reaction between concanavalin A and host components (8) a more compelling explanation for the sensitizing activity of this phytohemagglutinin involves the histamine and serotonin-releasing mechanism reported in experimental anaphylaxis in the mouse (9). The failure of D-glucose to inhibit sensitization suggests that the protein's carbohydrate binding sites may not be involved, since the precipitin reaction of concanavalin A with polysaccharides is inhibited by D-glucose (2). However, the monosaccharide may have been removed by a host metabolic process, or the concentration of D-glucose may have been too low to inhibit the interaction with cellular carbohydrates (4). In addition, there is no direct evidence that the antigen–antibody-like interactions between concanavalin A and host tissues are the result of these protein–carbohydrate interactions.

The ability of concanavalin A to induce histamine sensitization in mice adds to the novel character of this protein and the need to characterize the unusual pharmacologic properties of this phytohemagglutinin.

Summary. The jack bean phytohemagglutinin, concanavalin A, induced histamine lethality in CFW mice. Sensitization depended upon the dose of concanavalin A, with an LD_{50} of 0.5 mg. The sensitization was time dependent, reaching a maximum at 4 days and declining at 6 days. An inhibitor of concanavalin A-polysaccharide precipitin interactions, D-glucose, had no effect on the sensitization. Concanavalin A did not alter the sensitization of mice by *B. pertussis*.

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