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Inflammation Induced by Concanavalin A and Other Lectins[1] (38153)

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(Introduced by J. Salk)

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A large number of biological activities of concanavalin A (Con A) and other lectins have been described (1) including mitogenicity for lymphocytes, cytotoxicity, tumor-specific cell agglutination, glycoprotein and polysaccharide binding activity, and inflammogenicity. The inflammatory activity of Con A has been reported only in relation to studies primarily concerned with its cytotoxicity (2) or with its Arthus-type reaction inducing properties (3). In the present report we describe the use in small amounts of Con A and other lectins to induce an intense and reproducible inflammatory response in experimental animals. The response to Con A is more prolonged than the response to other lectins. This effect may be related to its slow rate of elimination from the site of injection.

Materials and Methods. Measurement of the inflammatory response. Con A (2x crystallized) was obtained from Miles Laboratories dissolved in saturated sodium chloride. Precautions were taken to prevent aggregation of Con A during the preparation of solutions of the appropriate concentrations in 0.15 *M* sodium chloride buffered at pH 7.2 by 10 m*M* potassium phosphate (PBS). Wheat germ agglutinin was purified from wheat germ lipase (Calbiochem) by the method by Burger and Goldberg (4). Affinity purified pea (*Pisum sativum*) lectin was the gift of I. S. Trowbridge. Bovine serum albumin (BSA) and carrageenan were obtained from Sigma Chemical Co.

The indicated amounts of lectins dissolved in 10 μl PBS were injected sc in the right hind footpads of groups of ten 20 g Balb/c mice from our own colony; 10 μl of PBS was injected into the left hind footpad. The specific swelling at the metatarsus was measured with dial gauge calipers (National Camera Supply Co.).

The antiinflammatory drugs acetylsalicylic acid (aspirin), indomethacin, and hydrocortisone were obtained from Sigma Chemical Co. and administered ip as a solution, or a finely divided suspension in PBS, 1 hr before treatment with a lectin. The edema was measured 6 hr later and compared with the edema in the footpads of a control group of mice that received an ip injection of PBS.

Rate of Elimination from the site of injection in mouse footpads. Con A and BSA were radioiodinated with iodine-125 (New England Nuclear) by the method of McConahey and Dixon (5). The rates of elimination of ^{125}I-Con A and ^{125}I-BSA from the feet of Balb/c mice were determined by injecting 25 μg of one of the labelled proteins in 10 μl PBS sc in the right hind footpads of groups of 50 mice. At periods after injection groups of three mice were sacrificed, the right hind feet were excised at the hairline, and counted on a Packard Model 3375 Gamma Scintillation Spectrometer.

Intra-articular injection of Con A. The knee joints of New Zealand white rabbits were chosen because of technical difficulties involved in performing an intra-articular injection in smaller animals. Left hind knees of rabbits were treated with a single intra-articular injection of 2 mg Con A in 100 μl saline, and the right hind knees as well as the knees from an untreated rabbit and a saline injected rabbit served as controls. The joints

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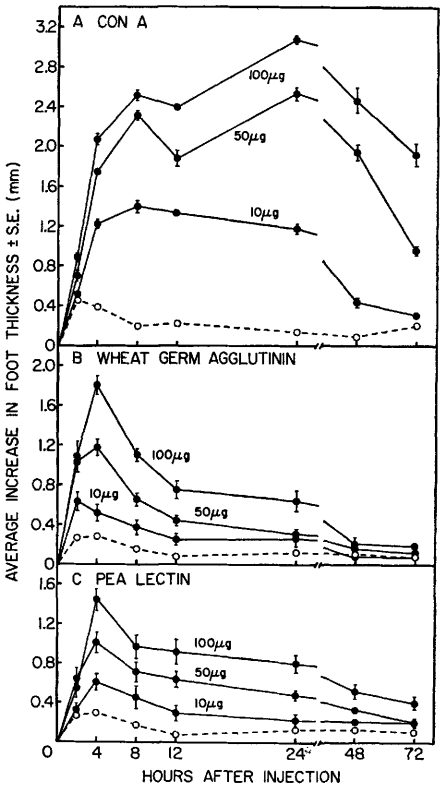

FIG. 1. Time course of the edema induced in the footpads of groups of 10 mice by (A) 100 μg carrageenan (--O--) and the indicated amounts of Con A (—●—); (B) 100 μg BSA (--O--) and the indicated amounts of wheat germ agglutinin (—●—); and (C) 100 μg of BSA (--O--) and the indicated amounts of pea lectin (—●—).

were examined after one day and at three weeks. Tissue samples for histology were taken from half of the rabbits at each of these times, fixed in 10% formalin, embedded in polyester, cut in 5 μm sections, and the sections stained with hematoxylin and eosin or with metachromatic stains.

Results. Inflammatory response to Con A and other lectins. As observed by More *et al.* (3), as little as 10 μg of Con A produces an intense edema with erythema and induration of the footpads of mice (Fig. 1A) with the maximum response at 8 hr and detectable edema remaining at 72 hr. Similar results were obtained with wheat germ agglutinin (Fig. 1B) and pea lectin (Fig. 1C). Higher doses of Con A (Fig 1A) cause an even more intense edema in which the feet of the mice swell to more than twice their normal thickness, which is about 2.6 mm. The response to Con A is prolonged, especially at higher doses which induce the development of a central necrosis at the site of injection several days later. The antiinflammatory drugs acetylsalicylic acid (aspirin), indomethacin, and hydrocortisone reproducibly inhibit the induction of edema (Table I). The nonsteroidal drugs administered orally also inhibited the inflammatory response.

Rate of Elimination of Con A from the Site of Injection. The basis of the prolonged reponse to injected Con A was investigated further by comparing the rates of elimination of ^{125}I-Con A and ^{125}I-BSA from the site of injection in the footpads of mice. ^{125}I-BSA, which lacks the saccharide binding properties of Con A (1), was eliminated much more rapidly than ^{125}I-Con A (Fig. 2), e.g., the time for 90% elimination was 48 times greater for ^{125}I-Con A than for ^{125}I-BSA. In neither case is the elimination of the protein a first order or even a unit order process, and a Powell plot (6) indicates that the elimination of Con A is a higher order process than the elimination of BSA.

Intra-articular injection of Con A. One possible application for a potent inflammogen that is retained at the site of injection for an unusually long period was investigated in rabbit knee joints. Within 6–8 hr after injection of Con A a local acute inflammation developed in the Con A treated knees with marked swelling, heat, and erythema. Twenty-four hrs after injection the treated joints contained elevated amounts of low viscosity synovial fluid which was heavily infiltrated with polymorphonuclear leukocytes (PMN, > 95%). Adipose tissue and the synovial membrane from the treated joints were also heavily infiltrated with PMN at 24 hr. No detectable cellular infiltration or microscopic destruction of cartilage was observed.

The joints were still swollen 3 weeks after treatment with Con A and the rabbits continued to limp favoring the swollen knee. The joints contained copious (8–10-fold the normal volume), low viscosity synovial fluid heavily infiltrated with both monocytes (approximately 40%), and PMN (45%–50%),

TABLE I. Effect of Antiinflammatory Drugs on Lectin Induced Inflammation in Mouse Footpads.

Lectin	Inhibitor	Inhibitor dose (mg/kg)	Average increase in foot thickness (mm) ± S. E. M.	% Inhibition of edema
Con A	Saline	—	1.08 ± 0.054	0
(10 μg)	Aspirin	50	0.89 ± 0.063	17
		100	0.84 ± 0.044	22
		200	0.63 ± 0.031	41
	Indomethacin	6.25	0.87 ± 0.077	19
		12.5	0.83 ± 0.044	23
		25	0.56 ± 0.044	48
	Hydrocortisone	25	0.86 ± 0.077	20
		50	0.60 ± 0.031	44
		100	0.35 ± 0.031	67
Wheat	Saline	—	0.84 ± 0.054	0
germ	Aspirin	50	0.73 ± 0.044	13
agglutinin		100	0.51 ± 0.031	39
(25 μg)		200	0.40 ± 0.044	52
	Indomethacin	6.25	0.69 ± 0.044	17
		12.5	0.43 ± 0.031	48
		25	0.33 ± 0.000	60
	Hydrocortisone	25	0.51 ± 0.000	39
		50	0.34 ± 0.000	59
		100	0.21 ± 0.031	75

suggesting a combination of both chronic and acute inflammation. A similar infiltration of adipose tissue and synovial membrane was observed as well as limited destruction of cartilage. There was no significant destruction of acid mucopolysaccharides detectable with metachromatic stains. Untreated joints of treated rabbits showed no evidence, relative to controls, of a sympathetic response even after 3 weeks.

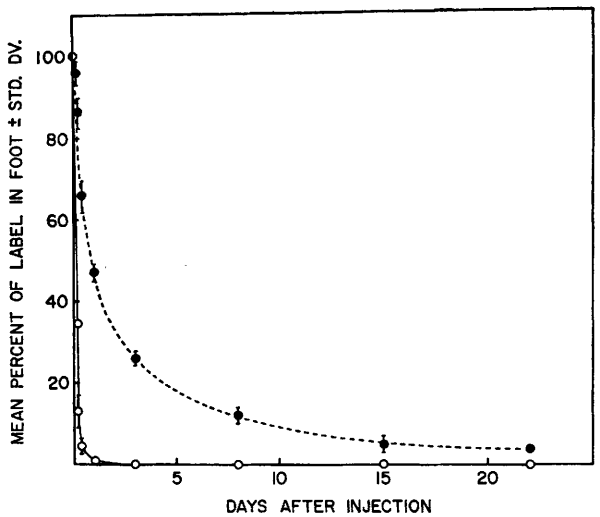

FIG. 2. Time course of the elimination of ^{125}I-Con A (--●—) and ^{125}I-BSA (—○—) from the site of injection in mouse feet.

Discussion. The results presented here suggest that mitogenic activity for lymphocytes is not essential to induce inflammation, since wheat germ agglutinin, which is not a mitogen for lymphocytes (7), induced essentially the same time course of inflammation as pea lectin, which is a mitogen (8). Since "skin reactive factor" is released by treating lymphocytes with mitogens over a 24 hr period (9), it is unlikely that it makes a major contribution to the inflammatory response to lectins, especially the initial response. The available data do not permit an assessment of the relative importance of precipitation of serum proteins [which has been suggested as an explanation for Arthus-type reactions to Con A in the skin of mice (10)], and cytotoxicity (2) [which may release the lysosomal enzymes that are found in elevated levels in inflammatory exudates (11)].

There are several possible explanations for the slow rate of elimination of Con A from the site of injection in mice. These include a mechanism involving binding of Con A to cell surface components of nonmotile cells, followed by internalization, so that the rate of elimination of Con A reflects the rate of turnover of these cells.

Many studies of inflammation have made use of injections of substantial quantities of carrageenin (12), a partially purified, poorly characterized galactan, or of croton oil (13) or turpentine, both of which are complex and undefined mixtures. The use of a purified agent such as Con A or other lectins, may be advantageous for some studies on inflammation. Because lectins induce a much more intense inflammatory response than carrageenin (12), reproducible measurements can be obtained readily without requiring a complex measuring apparatus. It would also seem that for studies of chronic as well as acute joint inflammation, a single injection of Con A provides advantages over procedures requiring multiple injections (14) which produce physical injury as well as specific damage.

Summary. Highly purified concanavalin A and other lectins injected in small amounts into the footpads of mice and rats induce an an intense and reproducible edema. Con A is eliminated from the site of injection at a slow rate, which may explain the prolonged nature of the inflammatory response it induces. Some advantages of the use of concanavalin A for the induction of experimental inflammation are discussed.

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