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Inhibition of Zymosan-Induced Alternative Complement Pathway Activation by Concanavalin A

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Zymosan, a polysaccharide composed primarily of glucan and mannan residues, activates the complement system through the alternative complement pathway. We showed that zymosan-induced complement activation is inhibited by zymosan-bound lectins with carbohydrate specificities for mannosyl and glycosyl residues. Lectins unable to bind mannosyl or glucosyl residues did not inhibit zymosan-induced complement activation.

The alternative complement pathway consists of six serum proteins, namely, C3, factor B, factor D, properdin, β1H globulin, and C3b inactivator. These components, in the presence of appropriate activating surfaces, interact to generate enzymes that activate C3 and C5 (4, 19). Agents known to activate the alternative pathway include certain immunoglobulins (1, 15), microorganisms (18, 23), mammalian cells such as rabbit erythrocytes (13), and zymosan (4, 12). Although the activity of zymosan is enhanced by immunoglobulin G antibodies in normal human serum (16), zymosan will activate the alternative pathway in the absence of immunoglobulins (4).

Zymosan is a polysaccharide obtained from yeast cell walls, and it is primarily composed of glucan and mannan residues (2). Since concanavalin A (ConA) binds to mannosyl and glucosyl residues (21), we undertook experiments to study the effect of ConA and other lectins on zymosan-induced activation of the alternative complement pathway. We showed that zymosan-induced activation of this pathway is inhibited by lectins that bind mannosyl or glucosyl residues or both, but that activation is not inhibited by lectins that do not bind these carbohydrates residues.

MATERIALS AND METHODS

Zymosan. Preparations of zymosan were obtained from Sigma Chemical Co., St. Louis, Mo.; Schwarz/Mann, Orangeburg, N.Y.; and Fleishman Yeast Div., Standard Brands, Inc., Stamford, Conn.

Lectins. Preparations of ConA (*Canavalia enisformis*) were obtained from Pharmacia Fine Chemicals, Piscataway, N.J.; Calbiochem, San Diego, Calif.; and Vector Laboratories, Inc., Burlingame, Calif. Soybean (*Glycine max*), wheat germ (*Triticum vulgaris*), lentil (*Lens culinaris*), garden pea (*Pisum sativum*), castor bean (*Ricinus communis* agglutinin 1), and gorse (*Ulex europaeus* agglutinin 1) agglutinins were obtained from Vector Laboratories, Pharmacia Fine Chemicals, and Miles Laboratories, Inc., Elkhart, Ind.

Human serum. Preparations of serum were obtained and stored as described previously (9).

Radiolabeled lectins. Insoluble lactoperoxidase (Bio-Rad Laboratories, Richmond, Calif.) and ^{125}I (IMS30; Amersham Corp., Arlington Heights, Ill.) were used to label (22) preparations of ConA (specific activity, 5 to 6 μCi/μg) and of garden pea (specific activity, 7 μCi/μg) and wheat germ (specific activity, 10 μCi/μg) agglutinins. Radiolabeled preparations were stored at −70°C, and radioactivity was measured with a Tracor Analytic gamma counter, model 1190 (Tracor Inc., Elk Grove Village, Ill.).

Measurement of radiolabeled lectin binding to zymosan. A 10-μl portion of the ^{125}I-labeled lectin preparation under study was mixed with 1.0 ml of unlabeled lectin preparation that contained 1.0 mg/ml. A 100-μl portion of this mixture was incubated with samples of 50 to 1,000 μg of zymosan at 22°C for 20 min. The zymosan was separated by centrifugation at 6°C for 5 min in an Eppendorf centrifuge 5412 (Brinkmann Instruments, Inc., Westbury, N.Y.). The zymosan pellets were resuspended in 1.0 ml of buffer and washed twice before counting. These experiments were performed in 1.5-ml polypropylene centrifuge tubes (Walter Sarstedt, Inc., Princeton, N.J.). Triethanolamine-buffered saline (pH 7.4; ionic strength, 0.15) containing 0.15 mM Ca^{2+} and 0.5 mM Mg^{2+} (9) and Veronal-buffered saline (pH 7.5) containing 0.15 mM Ca^{2+} and 0.5 mM Mg^{2+} (14) were used interchangeably in these experiments.

Assay for the effect of lectins on zymosan-induced activation of the alternative complement pathway. In these experiments, mixtures of zymosan and the lectin under investigation were incubated at 22°C for 20 min. The zymosan was separated by centrifugation and washed three times to remove unbound lectin. Zymosan pellets obtained from these experiments and control tubes containing the same amount of zymosan that had not been incubated with the lectin preparation were suspended in 100 μl of buffer, mixed with 400 μl of normal human serum, and incubated at 37°C for 45

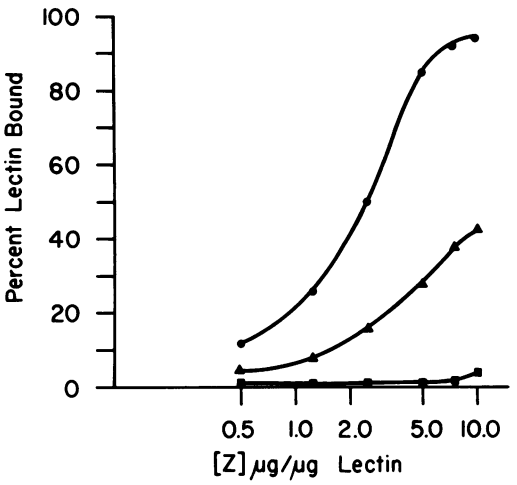

FIG. 1. Binding of lectins to zymosan. Percentage of ConA (●) and of garden pea (▲) and wheat germ (■) agglutinins bound to zymosan is plotted on the ordinate; zymosan concentration, expressed as micrograms of zymosan per microgram of lectin in each reaction mixture, is plotted on the abscissa.

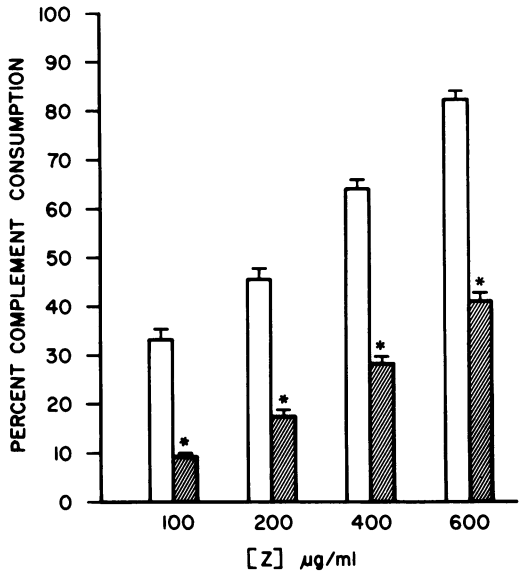

FIG. 2. Effect of ConA on alternative complement pathway activation by zymosan. Percentage of complement consumed during incubation with either zymosan alone or zymosan-ConA complexes is plotted on the ordinate; open bars represent reaction mixtures containing only zymosan, and cross-hatched bars represent reaction mixtures containing zymosan-ConA complexes. Zymosan concentration in each mixture is plotted on the abscissa. The values represent the mean ± the standard error of the mean for duplicate determinations. *, $P < 0.01$ when complement consumption in mixtures containing zymosan-ConA complexes is compared with consumption in mixtures containing only zymosan.

min. An additional control mixture of 400 μl of serum and 100 μl of buffer was included in each experiment. After incubation, the zymosan was separated by centrifugation, and whole complement activity in the supernatant serum was measured by immune hemolysis. These experiments were performed with either triethanolamine- or Veronal-buffered saline buffer.

Assays for whole complement and complement component activities. Whole complement, C1, and C3 activities were measured as described previously (9).

RESULTS

Binding of ^{125}I-labeled lectins to zymosan. The binding of ^{125}I-labeled ConA to zymosan was studied by incubating 100 μg of ConA with samples of 50 to 1,000 μg of zymosan. Under these conditions, ConA bound to zymosan in a nonlinear fashion, and the amount of ConA bound varied with the concentration of zymosan. Results from a typical experiment are shown in Fig. 1.

The binding of ^{125}I-labeled garden pea and wheat germ agglutinins to zymosan was investigated under conditions identical to those described above for ConA. Wheat germ agglutinin did not bind to zymosan (Fig. 1). On the other hand, garden pea agglutinin did bind to zymosan (Fig. 1), but the extent of binding was markedly diminished when compared with that of ConA, even though both lectins are specific for mannosyl and glucosyl residues (20). The limited binding of garden pea agglutinin to zymosan was not a spurious result associated with a particular zymosan preparation, since similar results were obtained with three different lots of zymosan.

Inhibition of zymosan-induced activation of the alternative complement pathway by ConA. After showing that ConA bound to zymosan, we investigated the effect of ConA on alternative complement pathway activation by zymosan. In these experiments, samples of 50 to 300 μg of zymosan were incubated with 20 μg of ConA, the zymosan was separated and washed by centrifugation and reincubated with normal human serum, and complement activity in the supernatant serum was determined as described above. Complement consumption was related to zymosan concentration in the reaction mixture, but consumption was reduced in mixtures containing ConA bound to zymosan (Fig. 2). Furthermore, inhibition by ConA of zymosan-induced complement consumption correlated with the amount of ConA bound to zymosan (Fig. 2). For example, complement consumption was inhibited by 72% in mixtures containing zymosan previously incubated with 0.4 μg of ConA per μg of zymosan (first bar in Fig. 2), but complement consumption was inhibited by only 54% in mix-

TABLE 1. Effect of zymosan-bound ConA on C1, C3, and whole complement activities

Zymosan (μg)	ConA (μg)	Activity[a] of:		
		Whole complement	C1	C3
250	50	56	150,000	7,106
250	25	48	145,000	5,858
250	12.5	39	155,000	4,258
250	0	29	154,000	3,390
0	0	67	157,000	7,920

[a] Activities are expressed as 50% hemolytic complement units per milliliter of serum. Normal values (± standard deviations) in this laboratory for these complement assays are: whole complement, 80 ± 20; C1, 190,000 ± 44,000; and C3, 8,100 ± 2,600.

tures containing zymosan previously incubated with 0.1 μg of ConA per μg of zymosan (third bar in Fig. 2). Complement consumption was not inhibited in serum that had been previously incubated with zymosan-bound ConA when it was reincubated with fresh zymosan. In addition, complement consumption was not inhibited when serum was incubated with zymosan from which ConA had been dissociated with α-methylmannoside.

To demonstrate that complement consumption in serum incubated with zymosan was primarily related to zymosan-induced activation of the alternative complement pathway, and to show that ConA did inhibit zymosan-induced activation of the alternative pathway, C1 and C3 activities were measured in serum incubated with zymosan alone and in serum incubated with zymosan to which ConA (zymosan-ConA complexes) had been bound. In these experiments, 250-μg zymosan pellets were incubated with buffer alone or with 12.5 to 50 μg of ConA for 20 min at 22°C, washed three times, and reincubated with 400 μl of serum at 37°C for 45 min, and total complement, C1, and C3 activities were measured in the supernatant serum. Under these conditions, total complement and C3 activities were markedly diminished in serum incubated with zymosan alone when compared with their activities in serum incubated without zymosan, but the activity of C1 was essentially the same in both mixtures. When zymosan-ConA complexes were incubated with serum, however, the reduction in whole complement and C3 activities was inhibited in a dose-dependent manner whereas C1 activity was not affected. Results from a typical experiment are given in Table 1.

Additional studies were performed to investigate the inhibitory effect of ConA on zymosan-induced activation of the alternative pathway by determining the effect of ConA on factor B activation. In these experiments, 250-μg pellets of zymosan were incubated with either buffer or 200 μg of ConA for 20 min at 22°C. The zymosan was separated and washed; zymosan-ConA complexes in one tube were then incubated with 100 μl of 0.3 M α-methylmannoside for 20 min at 22°C, and the zymosan was separated and washed. The zymosan pellets were then incubated with normal serum at 37°C for 45 min, and the immunoelectrophoretic behavior of factor B was determined. The immunoelectrophoretic mobility of factor B was altered in serum incubated with zymosan alone and in serum incubated with zymosan from which ConA had been dissociated with α-methylmannoside, but factor B conversion was markedly inhibited in serum incubated with zymosan-ConA complexes (Fig. 3). Whole complement activities (expressed as 50% hemolytic complement units per milliliter) in the serum samples subjected to electrophoresis (Fig. 3) were: well 1, 64; well 2, 25; well 3, 29; and well 4, 72.

Effect of other lectins on zymosan-induced activation of the alternative complement pathway. Preparations of ConA and of soybean, wheat germ, lentil, garden pea, castor bean, and gorse agglutinins were incubated with zymosan; the zymosan was washed and reincubated with se-

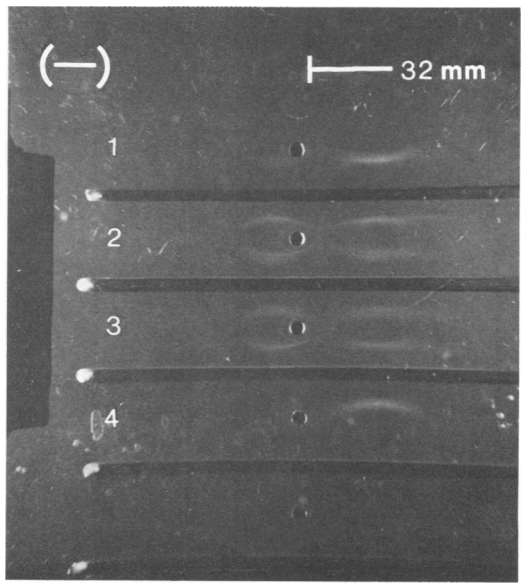

FIG. 3. Immunoelectrophoretic behavior of factor B in human serum. Well 1, 400 μl of serum incubated with 250 μg of zymosan-ConA complexes; well 2, 400 μl of serum incubated with 250 μg of zymosan from which ConA had been dissociated with α-methylmannoside; well 3, 400 μl of serum incubated with 250 μg of zymosan; well 4, 400 μl of serum incubated alone. A 5-μl portion of each mixture was electrophoresed and developed with anti-human GBG (factor B).

TABLE 2. Effect of lectins on zymosan-induced complement activation

Lectin	Amt of lectin (μg/μg of zymosan)	% Complement consumption	Carbohydrate specificity
ConA (*Canavala enisformis*)	0.4	8	α-D-Mannosyl, α-D-glucosyl
	0.2	11	
	0.02	50	
	0.0	68	
Garden pea agglutinin (*Pisum sativum*)	0.4	46	α-D-Mannosyl, α-D-glucosyl
	0.2	54	
	0.02	65	
	0.0	72	
Lentil agglutinin (*Lens culinaris*)	0.4	48	α-D-Mannosyl, α-D-glucosyl
	0.2	58	
	0.02	64	
	0.0	70	
Wheat germ agglutinin (*Triticum vulgaris*)	0.4	74	*N*-Acetylglucosaminyl
	0.2	74	
	0.02	73	
	0.0	72	
Castor bean agglutinin (*Ricinus communis* agglutinin 1)	0.4	72	D-Galactosyl
	0.2	70	
	0.02	72	
	0.0	74	
Soybean agglutinin (*Glycine max*)	0.4	72	*N*-Acetylgalactosaminyl
	0.2	71	
	0.02	67	
	0.0	66	
Gorse agglutinin (*Ulex europeus* agglutinin 1)	0.4	70	L-Fucosyl
	0.2	66	
	0.02	66	
	0.0	66	

rum, and whole complement activity in the supernatant serum was measured. Results from a typical experiment are given in Table 2. Complement consumption was inhibited in mixtures containing zymosan previously incubated with ConA or with garden pea or lentil agglutinins, but the other lectins had no effect on zymosan-induced complement activation. When compared with ConA, however, lentil and garden pea agglutinins were less effective as inhibitors of zymosan-induced complement consumption (Table 2).

DISCUSSION

Since zymosan is primarily composed of glucan and mannan residues (2), we undertook this study to determine whether lectins specific for these carbohydrate residues would bind to zymosan and inhibit zymosan-induced complement activation. ConA and garden pea agglutinin are specific for glucosyl and mannosyl residues (20). Both lectins did bind to zymosan, but compared with ConA, the binding of garden pea agglutinin was diminished (Fig. 1). On the other hand, wheat germ agglutinin, which is specific for *N*-acetylglucosamine residues (20), did not bind to zymosan (Fig. 1).

These lectins, together with others possessing carbohydrate specificities for *N*-acetylgalactosaminyl, L-fucosyl, and D-galactosyl residues, were tested for their ability to inhibit zymosan-induced complement activation. Lectins with specificities for mannosyl and glucosyl residues (i.e., ConA and garden pea and lentil agglutinins) inhibited complement activation, but zymosan-induced complement activation was not inhibited by the other lectin preparations (Table 2). Garden pea and lentil agglutinins were less effective than ConA as inhibitors of zymosan-induced activation, and their diminished activity correlated with their limited binding to zymosan.

Since these experiments were performed with whole human serum, it was necessary to determine whether zymosan-induced complement activation occurred through the alternative complement pathway under the experimental

conditions used in this study. The observation that C1 activity was not diminished in serum incubated with zymosan, whereas C3 and whole complement activities were markedly reduced, is consistent with zymosan-induced activation of the alternative complement pathway. The dose-dependent inhibition of ConA of zymosan-induced C3 and whole complement consumption (Table 1) indicates that ConA inhibited zymosan-induced alternative pathway activation. This conclusion is supported by the demonstration that ConA inhibited zymosan-induced activation of factor B (Fig. 3).

Although these experiments show that zymosan-induced complement activation is inhibited by lectins that bind to zymosan, the nature of this inhibitory effect has not been established. Other investigators have shown that immobilized ConA can bind all components of the classical complement pathway except C6, C7, and C9, and that fluid-phase ConA inhibits the hemolytic activity of C1, C2, C3, C5, and C8 (5, 8). Since we have shown that zymosan-bound ConA inhibited zymosan-induced C3 consumption (Table 1), the effect of ConA cannot be attributed to the binding of C3 by ConA. On the other hand, ConA might inhibit zymosan-induced complement activation by inhibiting C3b binding to zymosan by steric hindrance or by occupying C3b binding sites on zymosan.

Several investigators have shown that surface constituents modulate recognition by the alternative pathway of activating versus nonactivating particles (3, 4, 6, 11, 19). Zymosan, for example, is transformed from an activating particle to a nonactivating particle when heparin glycosaminoglycan is coupled to its surface (7), and sheep erythrocytes are transformed into alternative pathway activators when a soluble lipopolysaccharide is incorporated into the cell membrane (10). Surface constituents that maintain a particle as a nonactivator (heparin and sialic acid) appear to act by promoting the interaction between particle-bound C3b and the regulatory proteins (β1H and C3b inactivator), whereas surface structures associated with activating particles appear to restrict the action of regulatory proteins (7, 10). These experiments suggest that ConA may transform zymosan into a nonactivating particle by promoting the interaction between particle-bound C3b and the regulatory proteins.

Other investigators have shown that immunoglobulin G antibodies specific for zymosan enhance C3 and factor B depletion by zymosan (16). These antibodies are present in normal human serum and they increase the rate of alternative pathway C3 convertase formation, but they do not influence the action of the regulatory proteins (17). In view of this work, ConA may act by inhibiting the binding of immunoglobulin G antibodies to zymosan and thereby diminish zymosan-induced complement activation.

As an alternative explanation, the inhibition of zymosan-induced activation of the alternative pathway by lectins specific for glucosyl and mannosyl residues suggests that a serum factor with receptor sites for these residues might bind to zymosan as an early event in the activation of this pathway. This possibility is supported by previous work which has shown that ZBP, a serum protein bearing such receptor sites, is required to measure properdin by the zymosan assay and binds to zymosan in the absence of other serum factors (9). Thus, ConA might act by inhibiting the binding of ZBP to zymosan. Although additional work is necessary to define the nature of lectin inhibition of zymosan-induced alternative pathway activation, these experiments indicate that carbohydrate residues on the surfaces of bacteria and viruses may be of major importance as regulators of alternative complement pathway activation.

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