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Properdin System and Immunity

II. Interaction of the Properdin System with Polysaccharides

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Properdin (*1, 2*) in conjunction with Mg^{++} and serum cofactors resembling the components of complement (C′) kills or inactivates certain bacteria (*3*) and viruses (*4*) and participates in the lysis of abnormal red cells (*5*). The observation that zymosan (*6*), an insoluble carbohydrate complex derived from yeast cell walls, combines with the properdin at 17° to 37°C in the presence of C′ and Mg^{++} led to the discovery and isolation of properdin (*1*). The resulting properdin-zymosan complex (PZ) inactivates the third component of complement (C′3) at 37°C (*1*). This forms the basis for the assay of properdin. Present evidence, although not conclusive, indicates that the mechanisms of interaction of properdin with zymosan and of properdin with bacteria and other agents are similar. However, C′3 does not appear to be inactivated in some of these reactions.

Previous work (*7, 8*) on the parenteral administration of zymosan and *E. coli* cell walls into laboratory animals demonstrated that zymosan and the cell walls interact with properdin *in vivo* and alter the serum properdin levels of the test animals. Shortly after the injection of these materials, the serum properdin titers fall to a low level but may return to levels considerably above normal within a few days. There appears to be a relationship between these changes in serum properdin titers *in vivo* and the susceptibility and resistance to experimental infection (*7–9*).

In order to obtain a better understanding of the full importance and the mechanics of the properdin system, a search was made for compounds that interact with the properdin system both *in vitro* and *in vivo* (*10*). It will be seen in the following discussion that zymosan is not unique in its ability to form a complex with properdin *in vitro* and to alter properdin levels *in vivo* but that this ability is shared with many other high molecular weight polysaccharides or polysaccharide complexes and cell walls of many bacteria.

Bacterial Cell Walls and Toxins

The action of various bacterial cell walls (*8, 11*) on the properdin system *in vitro* has been investigated here, and some of the results are summarized in Table 1. It will be seen that cell walls from yeasts and both gram-negative and gram-positive bacteria complex with properdin at temperatures between 15° and 37°C. These complexes are insoluble and can be removed from serum by centrifugation at 4000 rev/min at 1°C in an International refrigerated centrifuge (PR-II). Active properdin can be dissociated from the cell walls in a medium of high ionic strength at slightly alkaline *p*H (*1*). With the exception of *E. coli* B, C′3 is specifically inactivated by the cell walls, suggesting the formation of a cell wall-properdin complex similar to PZ, which is then capable of interacting with C′3.

The action of two purified typhoid endotoxins and diphtherial toxin on the properdin system is presented in Table 1. It will be noticed that purified protein-free endotoxin #282 (O antigen) (*12*) removes properdin from serum but does not inactivate C′3. Partially purified endotoxin #AKD-B-3 also removes properdin that could be eluted from the properdin endotoxin complex in the usual fashion. The partial inactivation of C′3 by this endotoxin may be due to contaminating cell wall constituents. Diphtherial toxin, a classical exotoxin (*13*), does not combine with properdin. It should also be noticed that these toxins are anticomplementary at the levels tested and inactivate other components of C′.

Neutral Polysaccharides

Hestrin and coworkers (*14*) have shown that certain high molecular weight native levans and dextrans have infection-promoting activity when they are injected intravenously into mice that are infected intraperitoneally. From their work it appears that the infection-promoting activity of native levan and dextran is the result of an extraperitoneal interaction between these agents and the host. These experiments suggested the possibility that native levans and dextrans might interact with the properdin system *in vitro* and also alter serum properdin levels *in vivo*. Accordingly, a series of dextrans and levans, various zymosans and several other glucans have been tested. The results are summarized in Table 2, which shows the following.

1) Zymosans vary in their effect on the properdin system. Variations in method of preparation may lead to physical and chemical changes of these materials and cause differences in their activity. Zymosans like FL-1 and LE-1 are representative of those employed in this laboratory. Small amounts of these zymosans added to serum remove properdin and inactivate C′3. Zymosans like FL-145 do not inactivate C′3 but remove properdin, while IIFD is almost entirely inactive. It is obvious that care must be taken to employ suitable zymosans for studies on the properdin system.

2) Native dextrans showed variations in their ability to interact with the properdin system similar to those mentioned in a preceding paragraph for the zymosans. It will be noted that some dextrans combine with properdin and inactivate C′3, others only combine with properdin, and still others are inactive. However, none of the neutral polysaccharides were anticomplementary. The active compounds are all of high molecular weights. Low molecular weight clinical dextrans are entirely inactive. However, several

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Table 1. Effect of bacterial cell walls, polysaccharides, and toxins on the properdin system in human serum

Sample	Maximal amount added to each milliliter of human serum		Removal or inactivation of properdin (%)	Elution of properdin from P-sample complex	Inactivation of C'3 (%)	Anticomplementary or inactivation of other components of C'
	For removal of properdin (mg)	For inactivation of C'3 (mg)				
Cell walls						
E. coli CV	2	1	> 90	+	100	No
E. coli B	5	5	> 90	+	0	No
Rhodospirillum rubrum	2	5	> 90		100	No
Candida pulcherrima	2	5	> 90	+	100	No
Streptococcus faecalis	2	1	> 90	+	100	Yes
Micrococcus lysodeikticus	2	5	> 90		100	Yes
Bacillus megaterium	2	1	> 90	+	100	Yes
Bacterial toxins						
Typhoid endotoxin #282	1.5	3	> 90		0	Yes
Typhoid endotoxin #AKD-B-3	1.5	3	> 90	+	50	Yes
Diphtherial toxin (purified)	3	3	0		0	Yes
Cell wall polysaccharides						
Pneumococcus IV	3	3	50		75	Yes
Pneumococcus XIV	3	3	70		100	Yes
Pneumococcus VIII	3	3	0		0	Yes
Pertussis	2	5	> 90		0	No
Normal serum control			0		0	

Table 2. Effect of various neutral polysaccharides on the properdin system in human serum

Sample	Maximal amount added to each milliliter of human serum		Removal or inactivation of properdin (%)	Inactivation of C'3 (%)	Type AGU links*, †		
	For removal of properdin (mg)	For inactivation of C'3 (mg)			1,6 (%)	1,4-like (%)	1,3-like (%)
Zymosan LE-I	2	2	> 90	100			
Zymosan FL-I	2	0.5	> 90	100			
Zymosan FL-145	2	10	> 90	0			
Zymosan II-D	5	10	20	0			
Glucan (yeast)	5	5	> 90	0			
Glucan (Laminarin)	3	3	> 90	95			
Mannan (yeast)	5	5	0	0			
Clinical dextrans‡							
Cutter (M-1 Lot No. 5-8783B)	20	20	0	0			
Benger (Batch No. 2354)	20	20	0	0			
Glaxo (Batch No. 54/056)	20	20	0	0			
Native dextrans							
B1299S-3†	3	3	> 90	100	50	50	0
B1355S-4†	3	3	> 90	100	57	8	35
B1355-fraction S*	3	3	> 90	95	56	9	35
B1351-fraction L*	3	3	> 90	25	85	4	11

(*Continued on page 547*)

dextrans of high molecular weight are also inert, suggesting that molecular weight alone does not determine activity. No simple physical or chemical relationship exists between these compounds and their ability to bind properdin or inactivate C'3. There is some suggestion, however, that the degree of chain branching may be important. It should also be mentioned that, while the relative percentage of 1, 3 and/or 1, 4 linkages appears to be of importance in this respect, some differences have been found in behavior of dextrans that contain these linkages.

3) Native levans, with the exception of levan II (Hestrin), remove properdin almost completely from serum. It is interesting that levan II has little infection-promoting activity. However, all levans tested inactivate C'3. Properdin could be eluted from the levan-properdin complex in the usual manner.

4) A glucan prepared from yeast (*15*) behaves like FL-145, removing properdin alone from serum, while another glucan, Laminarin (*16*), combines with properdin and also inactivates C'3. A yeast mannan (*15*) is entirely ineffective in the properdin system.

It has been shown that antiserums to several pneumococcal polysaccharides cross-react immunologically (*17*) with some dextrans, suggesting similarities between the properties of these polysaccharides. Table 1 shows the results of the interaction of several pneumococcal polysaccharides with the properdin system. The polysaccharide from type XIV pneumococcus and to a lesser extent from type IV readily remove properdin from serum and inactivate C'3, while type VIII is not active against the properdin system. All of these compounds inactivate C'1, C'2, and C'4, indicating that complement fixation also occurs. This may account for certain peculiarities encountered in the assay of properdin and C'3 in the presence of these compounds. This, along with a lack of precise chemical and physical data for these agents, made it necessary to postpone further studies of the interaction of pneumococcal polysaccharides with the properdin system.

A carbohydrate isolated from *H. pertussis* (*18*) combines with properdin but does not inactivate C'3. This resembles the action of some of the afore-described dextrans.

Mucins and Ground Substance Components

It has been shown that the addition of mucins to serum *in vitro* (*19*) removed the bactericidal activity of serum and that the injection of mucin into guinea pigs resulted in a marked decrease

in the bactericidal activities of their serums. Therefore, it seemed important to determine the effect of several mucins on the properdin system. Crude hog gastric mucin (Armour) removes properdin and partially inactivates C'3. Another mucin (Rowley) combines with properdin but does not inactivate C'3. A mouse microsomal intestinal fraction (*20*) also combines with properdin. Other high molecular weight substances of mammalian origin are under investigation.

Reports in the literature (*21*) have suggested that certain rheumatoid diseases, diseases involving connective tissue or ground substance, and many inflammatory reactions may involve an immunological response of the host to the components of the ground substance. However, hyaluronic acid, the fundamental dimer of hyaluronic acid (*22*), chondroitin sulfate, heparin, and hyaluronidase (bull testes) are inactive on the properdin system (Table 3). None of these substances combines with properdin. Heparin is highly anticomplementary at the levels employed, making assays unreliable. The complete inactivity of the highly charged hyaluronic acid and chondroitin sulfate is surprising in view of the anticomplementary properties of heparin.

Requirements for Complement and Mg^{++}

Although kaolin and charcoal, as well as both anionic and cationic resins, do not combine with properdin, the possibility still existed that the removal of properdin from serum by the afore-mentioned agents was due to nonspecific adsorption and did not resemble the combination of properdin with zymosan, which requires the presence of C' and Mg^{++} (*1*). Accordingly, the requirements for the removal of properdin by levan, mucin, and endotoxin were studied. Purified properdin alone is not absorbed by these agents, as is shown in Table 4. The data also show that the inactivation of C' prevents the combination of properdin with these agents. It will also be noted that combination with properdin does not occur in the absence of Mg^{++}. Thus, C' and Mg^{++} are necessary for the combination of properdin, not only with zymosan, but also with other carbohydrates, carbohydrate complexes, and endotoxins.

Table 5 shows the requirements for properdin, C', and Mg^{++} in the inactivation of C'3 by levan. It will be noted that levan does not inactivate C'3 in the absence of Mg^{++}, C', or properdin. This is identical with the requirements for the inactivation of C'3 by zymosan (*1, 23,*

Table 2.—(*Continued*)

Sample	Maximal amount added to each milliliter of human serum		Removal or inactivation of properdin (%)	Inactivation of C'3 (%)	Type AGU links*, †		
	For removal of properdin (mg)	For inactivation of C'3 (mg)			1,6 (%)	1,4-like (%)	1,3-like (%)
B1146*	3	3	> 90	0	96	4	0
B1191*	3	3	> 90	0	71	14	15
B1254-fraction S*	3	3	> 90	0	93	7	0
B1402*	3	3	> 90	0	66	34	0
B742-fraction C-3R†	3	6	75	0	61	18	21
B1399-fraction L*	3	6	50	0	81	19	0
B1064*	3	3	50	0	95	5	0
B742-fraction L-R†	3	6	0	0	81	19	0
B1355I-C†	3	6	0	0	88	9	3
B1424*	3	6	0	0	72	28	0
B1375†	3	6	0	0	81	6	13
B512*	5	5	0	0	95	5	0
Native levans							
B1072*	3	3	75	100			
B512-E*	3	3	> 90	100			
Levan I§	2	1.5	> 90	100			
Levan II§	3	1.5	25	100			

* Samples and analytic data furnished by Allene Jeanes and F. R. Senti, Northern Utilization Research Branch, U.S. Dept. of Agriculture, Peoria, Ill. (*40*).
† Samples and analytic data furnished by Elvin A. Kabat, Columbia-Presbyterian Medical Center, New York, N.Y.
‡ Clinical dextrans furnished by M. H. Sloan, National Research Council, Washington, D.C.
§ Samples furnished by S. Hestrin, Hebrew University, Hadassah Medical School, Jerusalem, Israel.

Table 3. Effect of mucins, components of ground substances, and other agents on the properdin system in human serum

Sample	Maximal amount added to each milliliter of human serum		Removal or inactivation of properdin (%)	Elution of properdin from P-sample complex	Inactivation of C'3 (%)	Anti-complementary or inactivation of other components of C'
	For removal of properdin (mg)	For inactivation of C'3 (mg)				
Hog gastric mucin*	2	5	> 90		0	Yes
Hog gastric mucin†	3	3	> 90	+	50	Yes
Mouse microsomal intestinal fraction	3	3	> 90	+	0	Yes
Heparin	3	3			50	Yes
Hyaluronic acid (Na salt)	3	3	0		0	No
Hyaluronic acid (dimer)	3	3	0		0	No
Hyaluronidase	3	3	0		0	No
Chondroitin SO_4 (Na salt)	3	3	0		0	No
Pectic acid (Na salt)	3	3	0		0	No
Kaolin	10	10	0	−	50	Yes
Charcoal	10	10	0		0	No
Normal serum control			0		0	No

* Supplied through the courtesy of Derrick Rowley, Wright-Fleming Institute, London, England.
† Commercial product, Armour Laboratories, Chicago, Ill.

Table 4. Requirements for complement and Mg^{++} in the combination of properdin with hog gastric mucin, levan I, and endotoxin. All serum mixtures were incubated for 1 hour at 17°C. The samples were then centrifuged at 35,000 *g* for 1 hour, and the supernatants were tested for properdin activity.

Sample	Added properdin (units/ml serum)	Hog gastric mucin* (mg/ml serum)	Removal of properdin (%)
Normal serum		0	0
Normal serum		3	> 90
Properdin-deficient serum (RP)	6	0	0
Properdin-deficient serum (RP)	6	3	> 90
Veronal buffer alone	6	3	0
C'4-deficient serum (R4)		0	0
C'4-deficient serum (R4)		3	0
Resin-treated serum		0	0
Resin-treated serum		3	0
Resin-treated serum + Mg^{++}†		3	> 90
Resin-treated serum + Ca^{++}†		3	0

* Identical results were obtained with levan I and typhoid endotoxin.
† Final molarity of $Mg^{++} = 5 \times 10^{-4}$; of $Ca^{++} = 2.5 \times 10^{-3}$.

Table 5. Requirement for properdin, complement, and Mg^{++} in the inactivation of C'3 by native levan I (Hestrin). All serum mixtures were incubated for 1 hour at 37°C. The samples were then centrifuged at 35,000 *g* for 1 hour, and the supernatants were tested for C'3 activity.

Sample	Levan I (mg/ml serum)	Inactivation of C'3 (%)
Normal serum	0	0
Normal serum	3	100
Properdin-deficient serum (RP)	0	0
Properdin-deficient serum (RP)	3	0
Properdin-deficient serum (RP) + 3 units properdin	3	100
C'4-deficient serum (R4)	0	0
C'4-deficient serum (R4)	3	0
Resin-treated serum	0	0
Resin-treated serum	3	0
Resin-treated serum + Mg^{++}*	3	100
Resin-treated serum + Ca^{++}*	3	0

* Final molarity of $Mg^{++} = 5 \times 10^{-4}$; of $Ca^{++} = 2.5 \times 10^{-3}$.

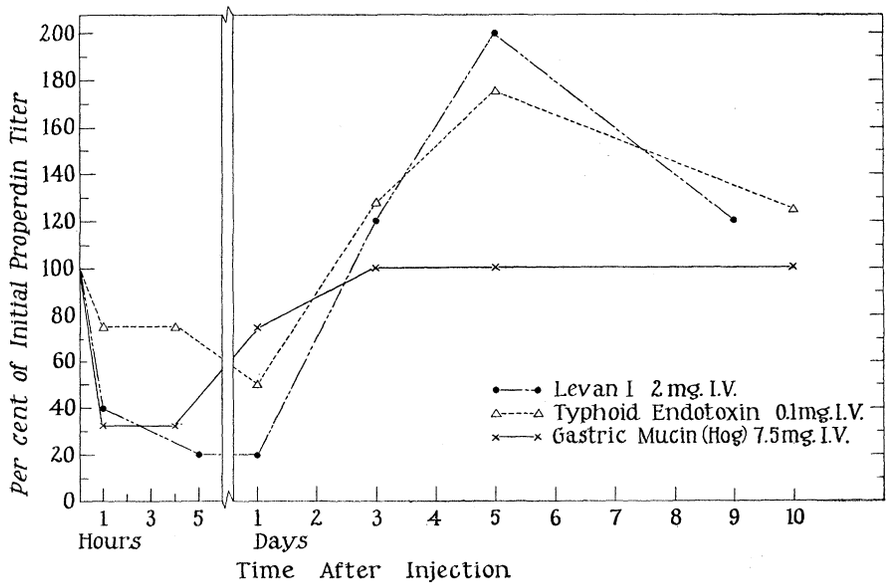

Fig. 1. The serum properdin levels in mice following intravenous injection of levan, mucin, and endotoxin.

24). The addition of properdin to properdin-deficient serum and the addition of Mg^{++} to resin-treated serum then allowed levan to inactivate C'3.

These experiments clearly show that the interaction of the properdin system with the afore-mentioned agents is dependent on the same conditions required for zymosan.

Possible Relationships to Natural Immunity

Most of the substances that have been shown to combine with properdin and to inactivate the properdin system have previously been demonstrated to have definite infection-promoting activity (*25*). These include dextrans and levans, hog gastric mucins, bacterial cell walls, and other bacterial carbohydrates.

Zymosan (*7*) and the cell walls of *E. coli* (*8*) have been shown not only to increase susceptibility but also to increase resistance to infection, depending on the time interval between the injection of these agents and the infection of the animal. Within the first few hours following the injection of zymosan or cell walls the animals are highly susceptible, whereas 2 to 5 days later they are resistant to various bacteria. Paralleling the degree of susceptibility of the animal to infection is a fall and rise in properdin levels during the same time interval, which suggests a relationship between serum properdin level and susceptibility or resistance to certain infections. The identical behavior of zymosan and the *E. coli* walls *in vivo* again suggests that they may contain similar compounds or structures.

It seemed important to determine the effect of levan, mucin, and endotoxin on the serum properdin levels *in vivo*. The results of such experiments are presented in Fig. 1, which shows that both levan and mucin produce a marked and rapid fall in properdin within a few hours after injection into mice; this is followed within 3 to 6 days by a return to normal levels in the mucin-treated mice and to a level of 200 percent of normal in the levan-treated mice. The injection of endotoxin caused a slight fall in properdin levels during the first 4 hours following injection, a more pronounced fall at 24 hours, followed by a marked increase in properdin levels between 3 to 6 days. All properdin levels had returned to normal values at about 10 days after the injections. Studies are in progress on properdin levels following the injection of varying amounts of these agents at different times.

The foregoing experiments suggest that the ability of mucins (*25–28*), levans (*14, 25*), and endotoxin to influence the resistance or susceptibility of laboratory

animals to infections may be due in part to their interaction with the properdin system. The interaction of endotoxins with properdin also suggests that the properdin system may be involved in "tolerance" to endotoxin (*29*), in the Shwartzmann reaction, and in the pyrogenic and other physiologic manifestations of endotoxins. The relationship of properdin to the normal serum factor (*30, 31*) that has been reported to react with endotoxin is under investigation. Along these lines, the increased sensitivity to endotoxins of dogs subjected to irreversible hemorrhagic shock (*32*), which is accompanied by a marked decrease in their serum properdin levels (*33*), should be noted.

Although a variety of high molecular weight carbohydrates or their complexes cause marked changes both in natural resistance and in the properdin system *in vitro* and *in vivo*, no definite correlation exists between the activities of these materials and any simple physical or chemical property. Polysaccharides with identical repeat units and very similar structures present widely different activities. The active materials contain both α- and β-linkages, furanosidic and pyranosidic units, and interhexose linkages of 1, 4; 1, 6; 1, 3; 2, 1; and 2, 6 types and combinations of these within the same compound. There also seems to be no dependence on the presence or absence of polar groups. Perhaps specific configurations or spatial arrangements of sugar residues may determine the ability of macromolecules, as well as certain bacteria, viruses, and red cells, to interact with the properdin system. In any event, the requirements of C′ and Mg^{++} for the combination of properdin clearly indicate that the mechanisms involved are highly complex and that further comment at this time would be highly speculative and premature.

Appendix

Nomenclature. Properdin is designated as P, complement as C′, and zymosan as Z. The four recognized components of C′ are indicated by the symbols C′1, C′2, C′3, and C′4 (*34*). RP indicates serum lacking properdin only and R3 lacking both C′3 and properdin. R4 is serum rendered free of C′4 by treatment with hydrazine (*35, 36*). The preparation of resin-treated serum that is deficient in Mg^{++} and Ca^{++} has been described previously (*37*).

General methods. Methods for the assay of properdin (*1*), C′, and C′ components (*1, 37, 38*) have been previously described. The various agents tested for their effect on the properdin system were dissolved or suspended before use to a concentration of 10 mg/ml in Veronal buffer (*39*) containing Mg^{++} and Ca^{++}. Desired amounts of these substances were then added to human serums previously selected for their ability to make satisfactory R3 or RP reagents by zymosan treatment. About 20 percent of human serums make suitable RP and only 10 percent make suitable R3. These mixtures, along with normal treated and untreated serum controls, were then incubated at 37°C for 1 hour with occasional mixing, then centrifuged at 35,000 *g* at 2°C for 1 hour, and the supernatants were tested for properdin, C′3 activity, and other C′ component activities. The presence of agents *that did not sediment at 35,000 g* interfered with both the properdin and the C′3 assays.

In certain instances, the afore-mentioned mixtures were incubated at 17°C instead of 37°C and treated as just described, and the supernatants were tested for properdin only. Some residues were eluted with buffer of *p*H 7.4 and ionic strength of 0.6, and the eluate was tested for properdin. Such tests verified the actual combination of properdin and eliminated the possibility of nonspecific inactivations.

To test the *in vivo* action of various agents on the properdin system, healthy 12- to 16-g CF2 female mice were injected intravenously with various doses of levan, mucin, and endotoxin dissolved or suspended in 0.15*M* NaCl. Changes in the properdin content of the blood were followed by doing titrations on pools of at least six serums obtained from groups of animals sacrificed at various times before and after injection. The serum samples were frozen and stored at −30°C in a mechanical deep freeze until the final sample had been obtained. Properdin titrations were then done on all samples with the same reagents on the same day.

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The truth is never simple, and rarely pure.—OSCAR WILDE.