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Properdin

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PROPERDIN

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INTRODUCTION

Resistance to infection is the sum total of a large number of interrelated factors which may affect the host-parasite relationship. These factors and their relationships are diverse and, as yet, incompletely defined. The extraordinary natural resistance of man and animals to infection has been pointed out by their spontaneous recovery and survival in a milieu of potentially infectious agents and by the relatively few infections sustained by them. The physical and chemical properties of skin, mucous membranes and their secretions, and the inflammatory response are among the major barriers to successful penetration of a host by infectious agents. Humoral factors are believed to play a role only after such penetration. These humoral factors of resistance have been intensively studied by many investigators, probably because of their ease of demonstration and ready availability.

The observation of John Hunter (1) over 150 years ago that fresh blood did not decompose as readily as other putrescible material lent considerable impetus to the study of the bactericidal properties of blood

and serum. From this original observation came the description of antibodies and the heat-labile substance, alexin or complement. Subsequent studies on antibody and complement have contributed immensely to our knowledge of immunity. However, these specific serologic factors are only one segment of the general phenomenon of serologic resistance to infection, and it has been recognized that naturally occurring mechanisms of humoral resistance are probably present. The nature and significance of these mechanisms have been less susceptible to study, however. The subject of this thesis, the properdin system, which consists of properdin, complement and magnesium ions (Mg^{++}) would appear to be one of such mechanisms concerned with natural resistance.

COMPLEMENT

Complement is a non-specific substance present in all normal sera, and is not increased in amount as a result of immunization. The complementary action of fresh, unheated serum would appear to depend on the interaction of four naturally occurring constituents. The best known activities of complement are those in which it acts in conjunction with specific antibody to effect hemolysis or bacteriolysis. All four components of complement are necessary for this action; the inactivation of a single component destroys the hemolytic or bacteriolytic properties of specific antibody and the remaining components of complement. These four components of complement, designated as C'1, C'2, C'3 and C'4, were originally discovered by the effects of certain physical and chemical agents on each portion. Thus, ammonia, hydrazine and many other primary amines inactivate C'4 with little effect on the other components. After absorption by yeast cells or the insoluble cell wall residue of yeast (zymosan), only C'3 will be inactivated.

Although a considerable amount of information is

available concerning the action of components of complement and the requirements for calcium ions and Mg^{++} in immune hemolysis (3, 4) the mechanisms of action of complement remains unknown. Recent studies indicate that the first component of complement (C'1) remains in serum as a proenzyme which may be activated to an esterase by antigen-antibody aggregates. (5, 6, 7) The role of this enzyme in immune hemolysis and bacteriolysis still remains to be determined, however.

THE IDENTITY OF PROPERDIN

The discovery of properdin was a result of studies on the mechanism of inactivation of C'3 by zymosan. It had been assumed that C'3 was adsorbed to zymosan, and on this basis, attempts were made to elute C'3 in a purified state from zymosan. These experiments were not successful. During these investigations, it was found that the inactivation of C'3 by zymosan was a complex phenomenon, requiring the presence of factors resembling the other components of complement (8) and also Mg++. (9) Later, it was shown that still another factor was required for this inactivation in addition to complement and Mg++. (10) This factor was subsequently called properdin, from the latin pro and perdere, meaning to prepare to destroy. The inactivation of C'3 had some of the characteristics of an enzymatic reaction since the loss of C'3 occurred only at temperatures above 20^{0} C. and at pH near 7. Moreover, the activity of C'3 was relatively little affected if the reaction was carried out at 15 to 17^{0} C. If fresh zymosan was added to serum treated previously with zymosan at these lower temperatures, no inactivation of

of C'3 occurred if incubated at 37° C. The combination of zymosan with properdin at 15 to 17° C. was found to be reversible, since properdin could be eluted from the zymosan complex. If serum is treated at these lower temperatures with zymosan, the addition of purified properdin to this serum allows fresh zymosan to inactivate C'3.

The interaction of properdin with zymosan and its ability to elute from zymosan in solutions of high ionic strength at an alkaline strength was the first method devised for the purification of properdin. By this method, preparations have been obtained in about a 2500-fold purification in yields of 40% to 80%. These preparations contained 900 - 1000 units of properdin per milligram of nitrogen. Normal sera contain approximately 4 - 10 units per milliliter. (11, 12, 13) The original method described above was modified by Pennel, et al. (12) using plasma collected from either acid-citrate-dextrose or resin collected blood. Rothstein and Pennel (14) have reported the purification of properdin from Fraction I of plasma eliminating the use of zymosan. Pondman and Prins (15) have obtained purified properdin utilizing ion-exchange methods. So

far, these preparations are heterogeneous and contain small amounts of a variety of serum activities. Nonetheless, these studies have indicated that properdin would appear to be associated with the alpha-one globulins and probably contributes to less than 0.05% of the total serum proteins.

Purified properdin has also been found to be immunologically heterogeneous. Antisera to properdin prepared by the injection of properdin preparations into rabbits, indicate that there are least five immunochemically distinct antigens. Cross-absorption experiments indicate that only one of these is properdin, since the other four antigens have been found to be present in serum depleted of properdin (RP). (16)

Data of Pillemer (17) and Isliker (18) indicate that properdin may undergo aggregation and dissociation and in this manner, resembles the macroglobulins.

Properdin is found in Fraction III-1 of serum or Fraction I of plasma. (11, 12) It is not a gamma globulin since patients with congenital agammaglobulinemia have been demonstrated to have normal properdin levels. (11, 19) Properdin differs from classical antibody in other ways. The combination of properdin with zymosan

requires serum factors, and this resulting complex inactivates the third component of complement. In contrast, the combination of antigen and antibody does not require serum factors, and the product of this interaction inactivates the first, second and fourth components of complement without altering the third component. In addition, properdin has a wide range of microorganisms and cells against which it is effective, whereas antibody has been found to be relatively specific in its action.

Some investigations have indicated that inactivation of complement by zymosan is similar to complement inactivation by immune precipiate. (20, 21) Subsequent work by Osawa and Muschel (22) has shown that the bactericidal action of normal serum requires the presence of a substance very similar to antibody. They have shown that normal serum in which specific antibody is absent has no bactericidal activity despite the presence of the properdin system.

Nelson (26) suggests that properdin is an antibody of relatively low specificity which is capable of combining with polysaccharides to fix the first, second and fourth components of complement.

In general, however, the variety of reacting substances, the requirements for the interaction, and the effect on complement of the interaction suggest that properdin is probably not an antibody but a distinct protein with multiple biologic activities.

Purified properdin alone is inactive. By itself it does not combine with zymosan, nor does it have any enzymatic activities. It is not one of the four recognized components of complement. Properdin can be removed from serum without altering any of the other previously recognized activities of complement. Clotting factors, iso-agglutinins, antibodies and enzymatic activities remain in serum which is essentially free of properdin. (17)

THE ASSAY OF PROPERDIN

On the basis of the observation by Pillemer et al. (10, 27) that zymosan combined with properdin to inactivate the third component of complement, an assay was devised for determining the amount of properdin in human serum. (11) Other methods of assaying properdin by activities other than the C'3 inactivation have been proposed. One of these is the bactericidal assay of properdin. (28) It has been found that with different concentrations of properdin, and constant amounts of a properdin deficient serum (RP), a graded bactericidal response is obtained. However, Wedgewood (29) found that specific antibody may interfere with this assay. The bactericidal action of the properdin system involves lysis of the organisms. In this action, the narrow range of temperature in which this occurs as well as the fact that properdin does not appear to be used up have suggested a similarity to an enzymatic reaction.

In 1956, van Vunakis (30) described the neutralizing action of fresh serum on T2r+ bacteriophage. The active substance in fresh serum was heat-labile and the activity of the serum was dependent on the four

recognized components of complement, on properdin and on Mg++. Later, Barlow and his associates studied the kinetics of the inactivation and devised an assay based on phage-neutralizing activity. (31, 32, 33, 34) This technique was used by Pernis and Turri (35) in the assay of properdin levels in human sera. Landy et al. (36) utilized this method to determine the interference of endotoxic polysaccharides in a serum pool with known neutralizing activity.

The Z-assay of Pillemer and the bacteriophage neutralization assay of Barlow (PhN_{50}) are the two methods commonly used in clinical investigation. Laurell and Reyn (36) in their comparison of these two methods found that the PhN_{50} assay tended to give higher values than the Z-assay, however, the values were neither proportional nor correlated.

THE BIOLOGIC ACTIVITY OF THE PROPERDIN SYSTEM

The properdin system has been found to possess numerous biologic activities. It has been implicated in the destruction of certain bacteria (38), the inactivation of some animal and bacterial viruses (29, 32, 39), the lysis of abnormal erythrocytes (40) and the killing of a protozoan, Toxoplasma gondii. (41, 42) The requirements for all of these activities of the properdin system have been found to be very similar.

It has been found that erythrocytes from patients with paroxysmal nocturnal hemoglobinuria as well as erythrocytes from normal individuals which have been treated with weak solutions of tannic acid, are hemolyzed by normal serum components. (39, 43) No antibody has been found to be involved in this reaction. Paroxysmal nocturnal hemoglobinuria is a rare acquired hemolytic anemia, in which the patient's cells are hemolyzed by his own or normal human serum. The reaction requires a temperature greater than 25° C. and has an optimum pH of 6.8, which is the same pH optimum for the combination of zymosan with properdin. The following observations suggest that the properdin system is required

for the hemolysis of these abnormal cells:

1. Serum deficient in properdin, but not in complement (RP), fails to hemolyze these cells.
2. Purified properdin from human, cow or hog serum specifically restores the hemolytic property of RP but by itself, is not hemolytic.
3. Sera from which any component of complement, or from which Mg^{++} has been removed, fail to hemolyze these abnormal erythrocytes. These sera do not become hemolytic with the addition of properdin; and
4. The titer of properdin in untreated serum corresponds to the hemolytic activity of that serum.

Tannic acid cells are hemolyzed by normal serum at 37^{0} C. in the presence of the properdin system. RP fails to hemolyze such cells, but the addition of purified properdin restores its hemolytic activity in the same manner as has been previously described for cells from patients with paroxysmal nocturnal hemoglobinuria. The amount of hemolysis of tannic acid treated cells is directly proportional to the amount of properdin added to the serum.

The bactericidal effect of properdin was first demonstrated with a strain of Shigella dysenteriae (28) which was rapidly killed when exposed to serum at 37° C. but not when exposed to a properdin-deficient serum. A mixture of RP and properdin, however, was bactericidal, although properdin alone was completely inactive.

In addition to Sh. dysenteriae, strains of Salmonella, Pseudomonas, Proteus, Paracolobactrum, Escherichia and Bacillus are sensitive to the properdin system. In general, susceptible strains of bacteria appear to be largely gram-negative. The degree of resistance or susceptibility to the properdin system varies from species to speces, and even within strains. (44)

The activity of the properdin system against an infectious agent appears to require all four components of complement. Removal of any one component results in loss of activity, the readdition of the missing component is followed by restoration of activity to the system. Wardlaw, et al. (45) found that serum rendered deficient in divalent cations, either by treatment with an ion-exchange resin, or by the addition of ethylene-diamine tetraacetic acid (EDTA) is devoid of activity. The readdition of magnesium ions to serum treated in

this manner restored activity. Calcium ions were found to have no effect, neither replacing nor enhancing the activity of magnesium. Many other cations have been tested and none except Mg^{++} will restore activity at physiologic concentrations.

There is some evidence that the inactivation of virus and the lysis of bacteria or abnormal erythrocytes are catalytic processes. It has been found that the number of bacteria killed or virus particles inactivated is directly related to the size of the inoculum. If this were a simple adsorption process, this would not be true. (28, 37)

Wedgewood, et al. (38) found that properdin is partially depleted in the interaction of the properdin system with infectious agents. They suggest that this may occur as a result of combination of properdin with by-products of the reaction (such as endotoxin released by the lysis of bacteria).

Pillemer and Ross (46) found that the parenteral injection of zymosan, which combines with properdin in-vitro, causes marked alterations in serum properdin levels in laboratory animals. Following injection of zymosan, serum properdin levels fall immediately,

followed by marked increases in titer 200 to 300 per cent above normal levels in 2 to 10 days. Alterations of the serum properdin levels in-vivo also appear to influence the course of some experimental infections (47) and the susceptibility of animals to the effects of total body irradiation. (10, 47) These investigators found that serum properdin levels were decreased following lethal doses of total body irradiation in rats and mice. The intravenous injection of purified bovine and human properdin into irradiated rats and mice significantly protected these animals.

Experimental infections in mice with Escherichia coli and Klebsiella pneumoniae also appear to show a relationship between properdin levels and the survival of infected animals. (47)

Rowley (48) found that small amounts of purified cell wals of a virulent strain of E. coli and large amounts of cell walls from an avirulent strain also inactivate C'3 and combine with properdin. The avirulent strain is more susceptible to the properdin system than the virulent strain. The ability of these extracts of cell walls to inactivate C'3 and combine with properdin resembles zymosan in their action.

INTERACTION OF THE PROPERDIN SYSTEM WITH POLYSACCHARIDES

The removal of properdin from serum by zymosan is reflected by a loss of bactericidal, erythrolytic and virus-neutralizing activities. (35, 50) Zymosan has been found to be only one of many substances which will interact with the properdin system. The factor common to all of these is their content of complex high molecular weight polysaccharides. These substances include natural dextrans and levans, endotoxins or lipopolysaccharides derived from yeasts; and similar substances obtained from normal and neoplastic tissues (including human erythrocyte stroma). Not all lipopolysaccharides interact with properdin in serum in a similar manner. Some interact with properdin and inactivate the third component of complement; others combine with properdin without such inactivation; still others appear completely inactive. (51, 52, 53) These substances have the ability to combine with properdin in-vitro and to influence properdin titers in-vivo. (46, 53)

These substances containing high molecular-weight polysaccharides are also capable of evoking the classic responses in the host usually associated with bacterial endotoxins, such as pyrogenicity, tumor necrosis and the Schwartzmann reaction. (54) Injection of these polysaccharides in large doses generally depresses the serum properdin levels; whereas smaller doses initially result in a depression of the level followed by increased levels. (46, 53, 55, 56) The variations in susceptibility to challenge infection concomitant with these changes have been previously described. (46) These results cannot be interpreted as showing that properdin exerts a protective effect in-vivo, however, since subsequent investigations have shown that boiled properdin as well as serum depleted of properdin also protect. (57, 58)

It is postulated that the decrease in serum properdin levels found in infections could be caused by inactivation of properdin following interaction with living or dead bacteria, or with endogenous polysaccharides released from them, or with endogenous polysaccharides released from

host tissues damaged by the infectious process.

PROPERDIN LEVELS IN DISEASE STATES

The levels of properdin in serum of patients having various neoplastic diseases have been studied. The first of these studies (59) maintained that normal levels of properdin are present in patients who have malignancies. Subsequent studies, however, maintain that properdin levels are depressed in many patients. (61, 60, 62) These workers reported that patients with advanced cancers had low levels of properdin. Moreover, these patients had difficulty in rejecting homografts of cancer cells readily with establishment of invasive growth, whereas normal individuals could reject homografts. They could find no other alterations in humoral and cellular defense mechanisms to explain these results. Almost one-half of patients with cancer who were studied by Rottino and Levy (60) exhibited levels of properdin in serum of less than 2 units per milliliter. In contrast, the patients with Hodgkins disease tended to have levels of properdin that were essentially within the normal range, although there were

significant numbers of patients with lower or higher levels than normal. The seven patients with lymphatic leukemia were found to have somewhat elevated levels. These results were again confirmed, 45% of patients with cancer were found to have properdin levels of less than 2 units per milliliter. These low levels were found in 5% of normal controls as well, but in comparison, 47% of patients with degenerative disease such as arteriosclerotic heart disease were found to have low properdin levels. The work of Myerburg (63) appears to confirm these results. He found that patients with diverse carcinomas tend to have significantly lower levels of properdin than do normal individuals.

Low properdin levels have also been found in irradiation illness (57, 64) hemorrhagic shock and rheumatoid arthritis. (65)

Barandum and Isliker (66) observed that properdin titers were low in patients with severe tuberculosis. They ascribe this decrease to an elevation in the alpha-two globulin fraction. Laurell and Reyn (67) in their comparison of the

Z-assay and PhN_{50} assay showed that 50% of patients with rheumatoid arthritis and cancer showed properdin titers of less than 1 unit as compared to 15% of normal sera. Their results showed that by utilizing the phage-neutralizing assay, properdin levels of arthritic and cancer patients were within the same range as for normal sera.

Herbut et al, (68) found that rats can be conditioned to accept transplants of the human colon carcenoma HR 132 by intravenous injection of zymosan. However, this conditioning is not as effective as that produced by exposure to 300r total-body irradiation. The administration of human properdin to irradiated animals increased properdin titers of their sera almost threefold; however, this did not restore their resistance to implants of HR 132. They conclude that the natural resistance of rats to the transplantable tumor is not mediated through the properdin system. In addition, other investigators (69) have shown that the cytotoxic activity of normal human serum on Ul2 and HeLa cells is not dependent on the presence of properdin, but appears relative to complement. They attribute the toxicity

of this serum to C'3, C'4 and possibly C'2.

The low properdin levels found in experimental shock, in irradiation illness or in patients with advanced cancer might well be accounted for by the release of endogenous polysaccharides from damaged host tissue.

CONCLUSION

The serum factor properdin was first demonstrated in 1954 by its ability to combine with zymosan to inactivate the third component of complement (C'3). It was subsequently found that properdin, in combination with C'3 and Mg^{++}, has a wide variety of activities in-vitro. Among these activities are the lysis of certain bacteria and abnormal erythrocytes, the inactivation of some viruses, and the ability to combine with polysaccharides from a variety of sources such as bacteria, yeasts and normal or neoplastic tissues. It would seem unlikely that such a system could exist in serum without some biologic function. The in-vivo demonstration of this function is difficult however, because of the nonspecific responses to injection of properdin, as well as the relative impurity of purified preparations. So far, the selection of the experimental animal, the nature of the injury, the proper choice of controls, and the relationship between the time of administration of properdin and the challenge infection have been the variables in the in-vivo response to properdin.

Perhaps the most challenging interaction of

properdin is that with substances from neoplastic mammalian tissues. The relationship between lowered properdin levels in patients with cancer as well as other chronic diseases is difficult to evaluate, since the relationship may well be an indication of total body response rather than being a determining factor in this response.

Further studies are needed to determined the role of properdin in natural resistance.

The field of immunology has always had an important role in the consideration of the body response to various disease states, especially those conditions caused by bacterial and viral organisms. Much of the basic understanding of the immunologic response of the body has been theoretical. Recent years has seen great strides made in more accurately defining various immunologic principles. The present day therapy has controlled many of the bacteriologic type of infections so that much more investigation is directed towards disease processes which in the past did not receive the attention due it. There is much though being given by investigators to the role of immunology to the conditions

which we refer to as "collagen diseases" and to cancer. Results of the investigations that are now being carried out are beginning to play a more important role in our thinking of the pathogenesis of these diseases. This thesis is a review of the literature on a subject which represents a very tiny facet of immunology. I have attempted to show the relationship of the subject to the problem of infection and to the problem of malignant disease. Though I cannot make any conclusions as to the specific role played by the properdin system in the etiology of these conditions, I hope I have stimulated the reader to take a closer look at this relatively confined system in the field of immunology in relation to its application to the problems of infection, collagen disease, and cancer.

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