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Research Article

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Hypocomplementemia of Membranoproliferative Nephritis

DEPENDENCE OF THE NEPHRITIC FACTOR REACTION ON PROPERDIN FACTOR B

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ABSTRACT Membranoproliferative nephritis in children is frequently associated with a hypocomplementemia produced at least in part by C3 breakdown mediated by a circulating anticomplementary factor known as C3 nephritic factor (C3NeF). C3 breakdown by this factor in vitro requires the presence of a pseudoglobulin cofactor and magnesium. The present study provides evidence that properdin factor B (C3 proactivator) is activated in the nephritic factor reaction and is the direct mediator of C3 breakdown by C3NeF. Depletion of factor B from mixtures of normal human serum (NHS) and plasma from a patient with membranoproliferative nephritis (MPP), either by heating or by immune equivalence absorption, blocks C3 breakdown by C3NeF. Addition of purified factor B to these mixtures restores the anticomplementary effect. When purified factor B is added to mixtures of MPP and purified C3, breakdown also occurs. Associated with the C3 breakdown is a change in the electrophoretic mobility of factor B from the beta to the gamma position, a shift which has been associated with cleavage activation of the molecule. Further, serum factor B levels are often low in patients with membranoproliferative nephritis and bear a rough inverse correlation with C3NeF levels. It thus appears that factor B is the previously described heat-labile C3NeF cofactor. Whether the C3NeF reaction proceeds via a pathway comparable to that activated by the cobra venom factor or via that activated by zymosan or inulin cannot be determined from the present data.

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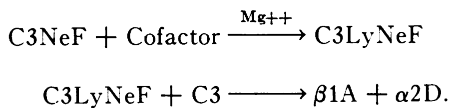
INTRODUCTION

Membranoproliferative glomerulonephritis (MPGN) [1] is a form of chronic nephritis which has a distinctive glomerular morphology and is frequently accompanied by hypocomplementemia (1–3). The serum of most of the hypocomplementemic patients contains an abnormal anticomplementary substance which is capable of breaking down the third component of complement, C3, when the serum is added to normal human serum (NHS) in vitro (4–7). The C3 breakdown does not require the presence of C1, C4, or C2. This anticomplementary substance, termed C3 nephritic factor (C3NeF), is a pseudoglobulin. Because serum containing C3NeF does not break down C3 added as euglobulin, it was postulated that C3NeF first interacts with a pseudoglobulin cofactor in NHS forming an active enzyme, C3 lytic nephritic factor (C3LyNeF), which then cleaved C3. The pseudoglobulin cofactor was found to be heat labile. Magnesium was necessary for the formation of C3Ly-

[1] *Abbreviations used in this paper:* C3LyNeF, C3 lytic nephritic factor; C3Nef, C3 nephritic factor; GBG, glycine-rich β-glycoprotein; MPGN, membranoproliferative glomerulonephritis; MPP, plasma from patients with membranoproliferative nephritis; MPS, serum from patients with membranoproliferative nephritis; NHS, normal human serum; RB, serum depleted of factor B; RBa-MPP, plasma with factor B inactivated by equivalence absorption; RBa-NHS, serum with factor B inactivated by equivalence absorption; RBh-MPP, plasma heated to inactivate factor B; RBh-NHS, serum heated to inactivate factor B; Z, zymosan.

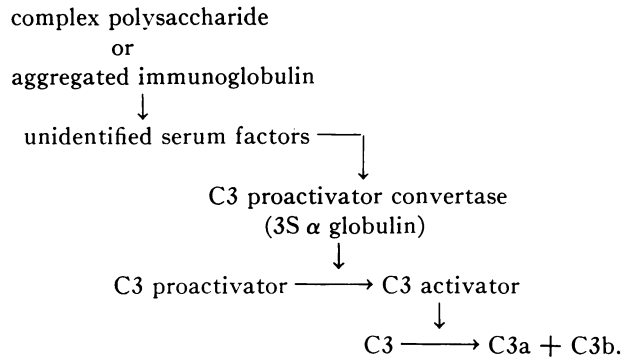
NeF. The postulated reaction sequence was as follows (4, 5):



Since C3 is the primary target of the C3 nephritic factor, the complement profile of patients with MPGN is distinctive and differs from that seen when immune complexes are added to NHS in vitro (8). In the latter instance, C3 convertase is formed from the early reacting components and all nine components are consumed to some degree. A similar profile is seen in the serum of patients with active lupus nephritis (9–11). In membranoproliferative nephritis, on the other hand, the C3 level is markedly reduced with sparing of the first three reacting components, C1, C4, and C2 (10–12).

Selective entry into the complement sequence at C3 has been previously described for other complement reactive factors. Selective interaction with C3 was a singular characteristic of the properdin system described by Pillemer et al. (13) in 1954. They reported that the yeast cell wall polysaccharide, zymosan, acting in conjunction with a hydrazine sensitive euglobulin (factor A), a heat sensitive pseudoglobulin (factor B), a second euglobulin they termed properdin, and magnesium, selectively broke down what was then considered to be the terminal complement component, C3. More recently, Gewurz, Shin, and Mergenhagen (8) demonstrated similar preferential C3 inactivation as well as inactivation of terminal components when a lipopolysaccharide endotoxin as well as zymosan was reacted with fresh guinea pig serum.

The mechanism which permits the bypass of the early reacting complement components has been recently delineated by Götze and Müller-Eberhard (14). This alternate pathway of C3 activation can be initiated by either complex polysaccharides such as zymosan, inulin, agar, and endotoxin (14) or by aggregates of certain human and guinea pig immunoglobulins (14–15). A hydrazine-sensitive protein is part of the early sequence which recent work by Müller-Eberhard and Götze (16) suggests may be C3 itself. Their studies of out-dated plasma suggest that a breakdown product of C3, tentatively identified as C3b, interacts with a 3S α-globulin to form an enzyme, C3 proactivator convertase. This enzyme cleaves a pre-enzyme, C3 proactivator, to form an enzymatically active fragment known as the C3 activator which in turn cleaves C3 to C3a and C3b. At this as well as at other steps in the reaction, further work may reveal the involvement of other proteins directly or as modifiers. However, currently the reaction sequence may be visualized as follows:



C3 proactivator is a heat-labile, hydrazine-resistant pseudoglobulin with a molecular weight of 80,000 which has a β-mobility by electrophoresis in agar (14). It has been considered identical to the glycine-rich β-glycoprotein (GBG) originally described by Boenisch and Alper (17) and has been found by Goodkofsky and Lepow to correspond to properdin factor B (18). For the remainder of this paper, the C3 proactivator will be referred to as factor B. The enzymatically active fragment, designated C3 activator, cleaved from factor B by C3 proactivator convertase, has a molecular weight of 60,000 and a γ-mobility on electrophoresis in agar (14).

The pathway leading to C3 breakdown by C3NeF could be comparable to that described above or, alternatively, could be comparable to the pathway activated by the cobra venom factor. In the latter pathway, the cobra venom factor is said to combine with a pseudoglobulin cofactor to form a C3 lytic factor which breaks down C3 without affecting the early reacting components. The original observations (19) gave evidence that the pseudoglobulin cofactor was the C3 proactivator which, as noted above, was later identified with factor B. However, subsequent studies by others (20) have not been able to identify factor B with the serum protein activating the cobra venom factor. Other investigators (21), on the other hand, have given evidence that factor B as well as two other proteins are necessary for the reaction in which cobra venom factor activates C3.

The present report provides evidence of a dependence of the C3NeF system as found in patients with MPGN on properdin factor B. The evidence is based on observations of serum factor B levels in patients with membranoproliferative nephritis and on the demonstration that in vitro factor B is not only necessary for lysis of C3 by C3NeF but also is cleaved during the C3NeF reaction to form the C3 activator fragment of γ-mobility.

METHODS

For the quantitation of C3, C3 nephritic factor and factor B levels in membranoproliferative nephritis, serum (MPS) was obtained from 14 patients who had histologic evidence of this type of nephritis by renal biopsy.

Plasma from a patient with membranoproliferative nephritis (MPP). All in vitro studies of the C3NeF reaction employed ACD plasma (anticoagulant citric acid dextrose solution) obtained by plasmaphoresis from one of the 14 patients mentioned above. The level of C3, C3NeF and factor B were quantitatively the same in the ACD plasma and the serum of this patient when corrected for the dilution occasioned by the ACD solution. In some experiments the plasma was first dialyzed overnight against 100 vol of 0.15 ionic strength, pH 7.6 phosphate buffer to remove the citrate. In other experiments enough magnesium as $MgCl_2$ was added to the undialyzed ACD plasma to provide free Mg^{++} in the reaction mixture.

Normal human serum (NHS). Serum obtained from normal volunteers was either used on the day it was drawn or stored at $-70°$ for later use.

Zymosan (Z). Zymosan (ICN Nutritional Biochemicals Div., Cleveland, Ohio) was boiled in 0.15M NaCl, centrifuged, and then resuspended in 0.15M NaCl at a concentration of 10 mg/ml. The zymosan suspension was stored at 6° until used. For activation of the alternate pathway, zymosan was added to NHS in the proportion of 10 mg/ml of serum.

C3. The euglobulin fraction of the serum of normal volunteers was subjected to TEAE cellulose chromatography using the method of Nilsson and Müller-Eberhard (22) to obtain partially purified C3. The preparation also contained C1, C5, and factor B. The concentration of factor B in the highly concentrated C3 preparations, as determined by radial immunodiffusion, was 40–50% of that of a pool of NHS. Since one part of the concentrated C3 preparation was usually added to 18 parts of the reaction mixture, the contribution of this preparation to the factor B content of the mixture was small. In some experiments, the purified C3 preparation was heated to 50° for 30 min to inactivate factor B (see below). This heat treatment was found to reduce to a slight extent the B antigen of C3 as measured by antibody consumption.

Serum depleted of factor B (RB). Two methods were used to remove active factor B from serum or plasma, heating to 50° for 30 min and absorption with monospecific antibody. Serum or plasma heated to inactivate factor B is designated RBh-NHS and RBh-MPP. Again, heating caused minimal reduction of the B antigen of C3. No factor B was detectable in the heated preparations by immunoelectrophoretic analysis or by radial immunodiffusion. Absorption by antibody at equivalence to produce an RBa-NHS and an RBa-MPP employed antiserum monospecific for factor B supplied as anti-β-glycoprotein II (Behringwerke AG, Marburg-Lahn, West Germany). The equivalence point was determined using a series of mixtures in which increasing quantities of NHS containing EDTA were added to constant volumes of antiserum. After appropriate incubation, the supernatants were removed by cold centrifugation and tested for unreacted antigen by double diffusion in agarose gel against the monospecific antiserum. Approximately 0.5 ml of antiserum was required to absorb 1 ml of NHS. The final RBa reagent was prepared in large volume by mixing serum and antiserum at equivalence as determined by the test run. After incubation and centrifugation, the RB supernatant was ultrafiltered to its original volume and the EDTA was removed by dialysis against phosphate buffer, 0.15 ionic strength, pH 7.6. By immunodiffusion, the RBa-NHS and the RBa-MPP had a concentration of factor B which was less than 10% of that in normal human serum.

Factor B. The method of Götze and Müller-Eberhard (14) was used to prepare partially purified factor B. The factor was detected in the column eluates by immunoelectrophoretic analysis using monospecific antibody (Behringwerke) as well as by conductivity. By electrophoresis in 7.5% polyacrylamide gel the factor B preparation was found to contain trace amounts of two contaminating proteins.

For some experiments highly purified factor B was obtained by cutting out the factor B segments from multiple unstained polyacrylamide gels. Factor B was eluted by stacking the corresponding gel slices in a dialysis sack (Visking 8/100-Union Carbide Corp., New York) filled with polyacrylamide reservoir buffer (tris glycine buffer, pH 8.2) and cross-electrophoresing the dialysis sacks for approximately 120 min in a lateral gel destaining cell filled with the same buffer. The sack buffer containing the factor B was then ultrafiltered and dialyzed against phosphate buffer, 0.15 ionic strength, pH 7.6. The purified preparation consisted of one protein band on repeat polyacrylamide gel electrophoresis and by immunoelectrophoretic analysis had a β-mobility when reacted with a monospecific antiserum to factor B.

Serum C3 determinations. β1A globulin, used as a measure of C3 (23), was quantitated by the immunoelectrophoretic-precipitin method (24). Before quantitation, the serum was aged at 37° for 5–7 days.

Serum factor B levels. Using antibody to factor B (Behringwerke), diluted 1:40 in agarose, factor B levels were measured in serum or reaction mixtures by single radial immunodiffusion (25). Standards consisted of appropriate dilutions of a pool of serum from 10 normal adults. Standards and unknowns were placed in wells in the agarose and allowed to diffuse overnight at 4°. The precipitin rings were then measured in two diameters at right angles using a 10× binocular microscope which had a reticule scaled in millimeters in the eye piece. The diameters were averaged and those of the standards graphed vs. their factor B concentration in percent of normal on semilog paper. From the linear relationship that was obtained, the factor B concentration of the unknowns was determined. Before quantitation each serum specimen was electrophoresed in agar and only specimens in which the factor B was in a β-position were used.

Measurement of C3 breakdown. The loss of the B antigenic determinant of C3 was used as a measure of C3 breakdown. The measurements were made either by the previously described immunoelectrophoretic precipitin method (5) or by a radial immunodiffusion method. The radial immunodiffusion method employed monospecific antisera to the B antigen prepared in goats incorporated in glycine-buffered agar, pH 7.0, which contained EDTA in a final concentration of 0.01 M. Standards consisted of normal human EDTA plasma in which the B antigen of C3 had been quantitated by the immunoelectrophoretic precipitin method. Appropriate dilutions of these standards as well as the unknowns were placed in wells and allowed to diffuse for about 18 h at 6°. After diffusion the slides were placed in 1% acetic acid for approximately 15 s to develop the precipitin ring. The rings were then measured in the same manner as described for the factor B quantitations. To quantitate the amount of C3 breakdown in an experimental reaction mixture, the following procedure was employed.

The reaction mixtures were made in an ice bath with all reagents at a temperature of 4° and a portion removed for measurement of the initial or zero time B antigen concentration. A second measurement of B antigen was made after appropriate incubation at 37°. The time of incubation was 20 min for the C3NeF reaction and 60 min for the zymosan reaction. The difference in the two B antigen concentrations was taken as a measure of C3 breakdown. Magnesium, as $MgCl_2$, was added to all reaction mixtures to a final concentration of 0.01 M.

Measurement of C3NeF. The method of measurement of C3NeF was described previously (26). The method cannot be used to detect levels of C3NeF below 15 U/ml since the standard curve does not go through the origin. The method is not highly reproducible for levels of C3NeF up to 35 U/ml and values in this range are of doubtful significance.

Mobility of factor B. Changes in mobility of factor B were detected by immunoelectrophoretic analysis of the serum mixture in 2% agar for 20 min at 10 V/cm. Monospecific antiserum to factor B was used to develop the arcs.

RESULTS

Concentrations of factor B, C3, and C3NeF in the serum of patients with MPGN. If, in vivo, C3NeF produces hypocomplementemia via the alternate pathway of C3 activation one might expect that as a result of utilization, serum factor B levels would be depressed in patients with MPGN. To test this possibility, measurements of C3, C3NeF, and factor B were performed on 23 serum specimens from 14 patients with MPGN. The C3 levels ranged from 6 to 50 mg/100 ml. As can be seen in Fig. 1, there was a rough inverse correlation between factor B and C3NeF levels. At high C3NeF levels, factor B was always significantly reduced whereas in specimens in which C3NeF was not demonstrable or was in low concentration, the factor B levels tended to be in the lower limit of the normal range.

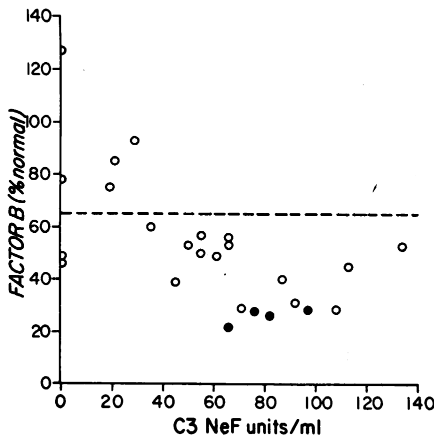


FIGURE 1 Serum factor B levels plotted vs. C3NeF levels for 14 patients with membranoproliferative glomerulonephritis. The closed circles represent values for specimens obtained from a patient with this disease who was anephric and was being maintained by hemodialysis.

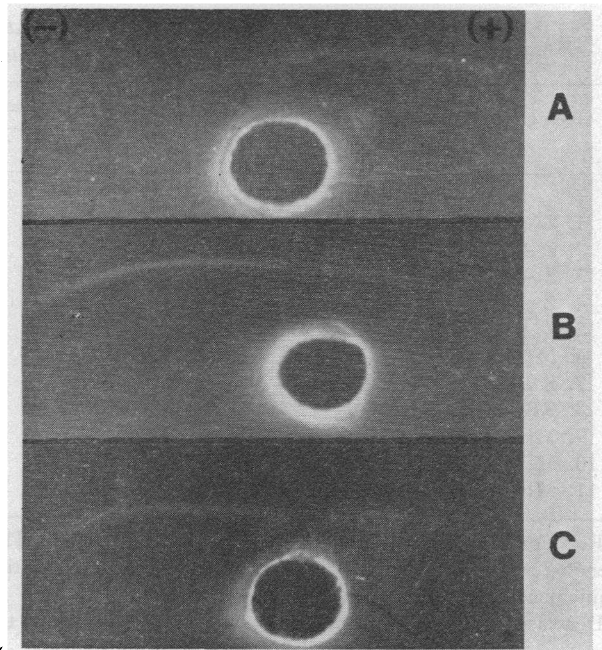


FIGURE 2 Effect of zymosan and MPP on electrophoretic mobility of factor B. Panel A is immunoelectrophoretic pattern of NHS incubated at 37° for 20 min, panel B is NHS after a reaction with zymosan at 37° for 60 min, and panel C, a mixture of MPP and NHS incubated for 20 min at 37°. In all cases the pattern was developed with a monospecific antiserum to factor B.

The correlation coefficient was 0.65. There was no correlation between serum factor B levels and urinary protein loss. Several of the nephrotic patients had normal factor B levels while other patients who were not nephrotic had reduced levels. In addition, as noted in Fig. 1, the lowest levels were found in a patient who at the time the specimens were obtained had been anephric for several months and was being maintained by hemodialysis.[2] Serum levels of factor B could not be correlated with the levels of C3.

Activation of factor B by MPP in vitro. If the C3NeF reaction produces C3 breakdown through cleavage and activation of factor B, the reaction would be expected to be associated with a change in the mobility of factor B from beta to gamma. To test this, portions of reaction mixtures of MPP and NHS and of NHS in which the alternate pathway was activated by zymosan were subjected to immunoelectrophoretic analysis using monospecific antiserum to factor B. As a control, immunoelectrophoretic analysis was done on a specimen of NHS incubated for 20 min at 37°. As shown in Fig. 2*A*, the factor B arc in this incubated control was in the typical β-position. After incubation of NHS with

[2] Serum factor B levels have been found to be within the normal range in other patients maintained by hemodialysis.

Table I

Effect of Depletion or Inactivation of Factor B on the Integrity of the Alternate Pathway and on the C3NeF Reaction

Reaction mixture*	B antigen		Decrease in B antigen	Factor B in mixture
	Zero time	After incubation		
	U/ml	*U/ml*	%	*% normal*
1. Z + NHS	21	9	57	55
2. Z + RBh-NHS	19	19	0	0
3. Z + RBa-NHS	28	28	0	5
4. Z + RBh-NHS + factor B	20	13	35	14
5. Z + RBa-NHS + factor B	30	25	17	36
6. MPP + NHS	21	11	47	79
7. RBh-MPP + NHS	20	13	35	73
8. RBa-MPP + NHS	23	14	39	74
9. MPP + RBh-NHS	19	19	0	6
10. MPP + RBa-NHS	34	31	9‡	8
11. MPP + RBh-NHS + factor B	25	13	48	30

* Reaction mixtures were made to a constant final volume. The MPP in mixtures 6–11 constituted 25% of the volume. RBh denotes serum or plasma with factor B inactivated by heating. RBa denotes serum or plasma with factor B removed by equivalence absorption.

‡ Decreases in B antigen of 10% or less at this level are within the range of error of the method and are considered insignificant.

zymosan for 60 min at 37°, there was a change in mobility of factor B from the β- to the γ-position (Fig. 2*B*) as would be expected with activation of the alternate pathway. The shift was associated with a 62% decrease in the concentration of the B antigen of C3, indicating significant C3 breakdown. A similar shift in mobility occurred when one part of MPP was incubated with three parts of NHS for 20 min at 37° (Fig. 2*C*). During this incubation there was a 47% decrease in the B antigen concentration. In all cases, immunoelectrophoresis of the reaction mixtures before incubation showed factor B to be in the β-position.

Requirement of the in vitro C3NeF reaction for factor B. To study the requirement of the C3NeF reaction for factor B, reaction mixtures were employed containing serum depleted of the pre-enzyme by heating and by equivalence absorption with monospecific antiserum. Zymosan (Z) was used to test the integrity of the alternate pathway in specimens of normal serum so treated. In Table I, it is apparent from reaction mixtures 1, 2, and 3 that a reduction in B antigen of C3 by 57% produced by addition of zymosan to NHS, failed to occur in serum specimens in which factor B was inactivated by heating (RBh-NHS) or removed by equivalence absorption (RBa-NHS). The anticomplementary activity of the zymosan is partly restored when purified factor B is added to the reaction mixture (mixtures 4 and 5). As will be discussed below, the incomplete restoration of activity may be because of the relatively low factor B levels in the mixture.

Inactivation of the factor B in MPP either by heating (RBh-MPP) or by equivalence absorption (RBa-MPP) reduced only slightly the ability of the MPP to produce C3 breakdown. As can be seen in Table I, untreated MPP, on addition to NHS, produced a 47% reduction in the B antigen of C3 (mixture 6) while an RB of the same MPP made by heating reduced the B antigen by 35% (mixture 7) and an RB made by absorption, by 39% (mixture 8). Because C3NeF activity is still demonstrable after MPP is treated to remove or inactivate factor B, it would appear that C3NeF is not identifiable with factor B.

In contrast to the lack of effect of depletion of factor B from MPP, similar depletion of NHS blocked C3 breakdown. Thus, breakdown was insignificant when MPP was added to an RBh-NHS or an RBa-NHS (mixtures 9 and 10). However when purified factor B was added back to the reaction mixture as shown in mixture 11, definite C3 breakdown occurred comparable in extent to that in the control mixture (mixture 6). It should be noted that although no factor B was added to mixtures 9 and 10, it was nevertheless present in concentrations 6–8% of that in normal serum as a result of the low factor B level in the MPP. The lack of C3 breakdown by C3NeF in mixtures containing low levels of factor B and the restoration of C3NeF activity on addition of factor B to the reaction mixture suggests that factor B is an integral part of the reaction mechanism of C3NeF.

To verify that factor B, rather than a contaminant in the partially purified factor B preparation, was essential for the C3NeF reaction, reaction mixtures were made containing eluates of sections of polyacrylamide gels cut as shown in Fig. 3. By immunoelectro-

phoretic analysis, section A was found to contain the contaminants and section B, the purified factor B. Section C, containing no protein, was used as a control. The eluates of these sections were added to mixtures containing RBh-MPP and heated purified C3 preparations. The only source of factor B in the mixtures was that in the eluates. As seen in Table II, no significant C3 breakdown occurred in mixtures containing eluates of sections A and C (mixtures 12 and 15). Eluates of section B, on the other hand, allowed significant C3 breakdown (mixture 13). The eluate was not active in the absence of a source of C3NeF (mixture 14). The results indicate that factor B, rather than a contaminant in the partially purified preparation, is essential for the C3NeF reaction and give further evidence that factor B is the only heat-labile factor necessary for the C3NeF reaction.

To assess the influence of the concentration of factor B and of C3NeF on C3 breakdown, MPP was mixed with purified C3 and factor B in varying proportions. As seen in mixture 16 of Table III, when MPP constituted 25% of the total volume of the reaction mixture, there was no C3 breakdown as would be predicted from previous studies (4, 5). This mixture had 11% of the normal level of factor B, furnished principally by MPP with a small amount contributed by the purified C3 preparation. When the factor B concentration was raised to 29% of normal by the addition of purified factor B (mixture 17), significant C3 breakdown occurred. This breakdown was not produced by the factor B preparation as evidenced by the insignificant reduction in B antigen in mixture 18 in which factor B was reacted with C3 in the absence of MPP. When the proportion of the reaction mixture constituted by MPP was increased to 50 and 75% and the concentration of substrate (C3) held constant, significant C3 breakdown occurred without further addition of factor B (mixtures 19 and 20). Thus with the substrate (C3) concentration held relatively constant, either the addition of factor B to the reaction at a low C3NeF concentration (mixture 17) or an increase in the concentration of C3NeF in the mixture relative to that of factor B (reactions 19 and 20) resulted in significant C3 breakdown.

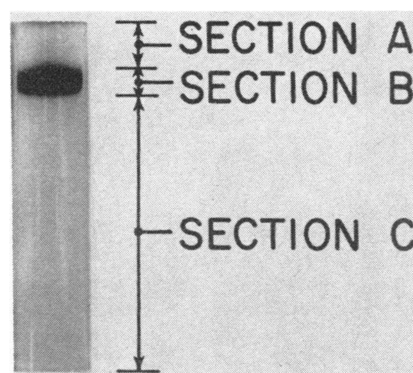


FIGURE 3 Acrylamide gel electrophoresis of a partially purified factor B preparation. Eluates of sections A, B, and C were used in reaction mixtures listed in Table II.

The requirement for factor B is further demonstrated in mixture 21 in which RBh-MPP providing C3NeF in high concentration (75% of in vivo level) is ineffective in producing C3 breakdown in contrast to the breakdown occurring in a similar mixture containing MPP which is not heat inactivated (mixture 20). The failure of C3 breakdown in mixture 21 can be attributed to the very low concentration of factor B in the mixture, since the activity of C3NeF is not destroyed by heating (mixture 7, Table I). Furthermore, addition of purified factor B (mixture 22) restores the anticomplementary activity of the RBh-MPP. Of interest is the difference in B antigen reduction in the two reaction mixtures with final factor B concentrations approximately 18% of normal (reactions 19 and 22). The difference in the extent of C3 breakdown in these two

TABLE II

C3 Breakdown in Mixtures in which the Sole Source of Factor B is a Highly Purified Preparation

Reaction mixture*	B antigen		Decrease in B antigen	Factor B in mixture
	Zero time	After incubation		
	U/ml	*U/ml*	%	*% normal*
12. RBh-MPP + Sect. A + C3h	38	38	0	0
13. RBh-MPP + Sect. B + C3h	32	23	28	20
14. 0.15 M NaCl + Sect. B + C3h	32	31	3‡	20
15. RBh-MPP + Sect. C + C3h	35	35	0	0

* Reaction mixtures were made to a constant final volume. RBh-MPP constituted 25% of the volume in mixtures 12, 13, and 15. C3h signifies purified C3 heated to 50° for 30 min. Sects. A, B, and C are eluates of sections of the polyacrylamide gel depicted in Fig. 2.

‡ Decrease of B antigen of this magnitude is considered insignificant.

Table III
Effect of Varying Concentration of C3NeF and factor B on C3 Breakdown

Reaction mixtures*	B antigen		Decrease in B antigen	Factor B in mixture
	Zero time	After incubation		
	U/ml	U/ml	%	% normal
16. MPP (25%) + C3	19	19	0	11
17. MPP (25%) + C3 + factor B	23	12	48	29
18. C3 + Factor B	25	24	4‡	20
19. MPP (50%) + C3	19	14	26	18
20. MPP (75%) + C3	18	7	61	24
21. RBh-MPP (75%) + C3	15	15	0	3
22. RBh-MPP (75%) + C3 + factor B	17	4	76	18

* All reaction mixtures were made to the same final volume with 0.15 M NaCl. Percentages in parenthesis indicate proportion of volume of mixture constituted by MPP.
‡ Decrease of B antigen of this magnitude is considered insignificant.

mixtures may be explained by the differences in the proportion of MPP and hence of C3NeF added to the mixtures.

DISCUSSION

Prior to the description of the alternate pathway of complement activation, several lines of evidence had indirectly indicated that the complement reaction in MPGN differed from that in other hypocomplementemic nephritides. A distinctive complement profile, similar to that seen when endotoxin or zymosan was added to NHS in vitro (8), was noted in the serum of patients with MPGN by several investigators (10–12). The complement profile fitted well with the subsequent observation that the serum of these patients often contained a circulating agent, C3 nephritic factor, which, when added to NHS in vitro, caused C3 breakdown without requiring the participation of C3 convertase (4, 5). A pseudoglobulin cofactor and Mg^{++} were demonstrated to be necessary for C3 breakdown by C3NeF.

The data presented here provide three lines of evidence that factor B is involved in C3 breakdown by C3NeF. First, factor B levels in the serum of hypocomplementemic patients with MPGN were reduced and tended to show a rough inverse correlation with C3NeF levels. Although urinary loss of factor B was not measured in these patients, the observations gave no evidence that it is of importance in producing the reduced levels. Second, incubation of mixtures of MPP and NHS resulted in a shift in the electrophoretic mobility of factor B to the γ-position, a shift which is associated with activation of the molecule in studies utilizing inulin or zymosan (14). This change in mobility was divalent cation dependent and did not occur with similar incubation of NHS in the absence of MPP. Finally, factor B was demonstrated to be necessary for C3 breakdown by C3NeF as indicated by the observation that removal of factor B from the reaction mixture either by heating or by immune equivalence absorption resulted in a block of C3NeF activity. Restoration of factor B to the reaction mixture, either as a semipurified or highly purified preparation, restored the ability of C3NeF to break down C3. The C3NeF itself was relatively unaffected by the manipulations employed to remove factor B. It thus appears that factor B is identifiable with the heat labile, pseudoglobulin cofactor for the C3NeF reaction which was described previously (4, 5). The observations obviously do not rule out other heat stable factors which could be critical for the C3NeF reaction nor do they clearly indicate whether the reaction pathway for C3NeF is that activated by addition of zymosan or inulin to serum or that activated by the cobra venom factor. Such a determination must await the availability of a purified preparation of C3NeF.

The observations presented here, are of interest in considerations of in vivo complement breakdown. Previous studies have indicated that the amount of C3 breakdown by the C3NeF system is dependent not only on C3NeF concentration but also on the concentration of C3 (5). Specifically, those studies showed that in reaction mixtures in which the C3 concentration was in the range normally present in plasma (B antigen 22–50 U/ml), a large amount of C3 was broken down and the extent of breakdown could be linearly related to the concentration of C3NeF in the mixture. At low C3 concentrations (B antigen 9 U/ml), there was little breakdown and that which did occur could not be correlated with the C3NeF concentration. Thus it might be predicted that at the low C3 concentration found in hypocomplementemic patients with membrano-proliferative nephritis (B antigen concentrations as low as 1.5 U/ml),

little C3 breakdown would be occurring even at high concentrations of C3NeF and adequate concentrations of factor B. If the steady state in the markedly hypocomplementemic patient were disturbed by administration of large quantities of C3, as was done in vitro in mixtures 19 and 20 of Table III, C3 breakdown would presumably be accelerated.

These observations provide some insight into the results of studies of Alper and Rosen (27) and of Peters et al. (7) of C3 metabolism in membranoproliferative nephritis. In hypocomplementemic patients with this disease, both groups found the rate of disappearance of radio-labeled C3 and the fractional catabolic rate of C3 to be the same as in the normal subject. Even though C3NeF was demonstrable in these patients (7), the calculated absolute catabolic rate of C3 was very low and, because they were in the steady state, the synthetic rate was perforce also low. A low rate of C3 breakdown as the result of a low C3 level would be predicted from the in vitro studies mentioned above (5). They would also predict that rapid catabolism would be demonstrable if sufficient C3 were administered with the radio labeled material to significantly increase the C3 plasma level. Thus these patients would have the potential for rapid catabolism of C3 and probably develop their hypocomplementemia as a result of breakdown via the C3NeF system. Once the hypocomplementemic state is achieved, the labeled C3 studies would indicate that diminished synthesis is a large factor in maintaining it. The mechanism responsible for the diminished synthesis is not known.

Remaining to be explained is the presence of α2D in the circulation of the hypocomplementemic patient in the steady state. This breakdown product of C3 has been demonstrated to be present in small amounts preformed in the circulation in the moderately to severely hypocomplementemic patient and to be absent in normal subjects (24, 28, 29). Although its presence has been considered an indication of accelerated C3 breakdown, an alternative and more correct interpretation could be that in the hypocomplementemic patient, the majority of the C3 breakdown which is occurring is via a mechanism which has α2D as an end product. In membranoproliferative nephritis, this mechanism would be the C3NeF system and by activating as well as breaking down C3, would account for the low levels of terminal complement components (7, 30). Whereas the β1A formed from C3 breakdown by this mechanism is apparently rapidly cleared from the circulation, as is C3i (27, 31), the α2D accumulates. α2D is not demonstrable in the normal subject because there is no circulating C3 lytic mechanism and catabolism of C3 is occurring by a mechanism which does not result in entrance of α2D into the circulation.

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