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Noorbibi K. Day, ... , A. Michael, R. A. Good

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Research Article

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The Association of Respiratory Infection, Recurrent Hematuria, and Focal Glomerulonephritis with Activation of the Complement System in the Cold

NOORBIBI K. DAY, H. GEIGER, R. MCLEAN, J. RESNICK, A. MICHAEL, and R. A. GOOD

From the Departments of Pathology and Pediatrics, University Hospitals, University of Minnesota, Minneapolis, Minnesota 55455

ABSTRACT The study of the activation of C3, C5, and C7 associated with the conversion of C3 in the serum of a 9-yr old girl after incubation at 0°C for 6–8 h without utilization of C1, C4, and C2 is described. The patient has upper respiratory infections associated with recurrent gross hematuria, focal glomerulonephritis, and transient renal insufficiency. Histological lesions demonstrated the presence of B1c globulin IgA and properdin in the glomeruli. The activation of complement (C) in the cold requires the patient's IgA. Removal of IgA from the serum by immunoadsorption prevents activation and conversion of C3. Bactericidal and phagocytic activity is also impaired after incubation. C3 proactivator (C3PA) level is reduced before and after incubation. Properdin level drops after incubation. These findings suggest that the activation of C3 which is demonstrable in vitro may be a continuous process in vivo.

INTRODUCTION

The activation of the complement (C) system by the alternate pathway whereby the earlier components C1, C4, and C2 are not involved is gaining increasing importance. This pathway involves a serum factor recently delineated by Götze and Müller-Eberhard as the C3PA (1). Goodkofsky and Lepow (2) have linked

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the alternate pathway with the properdin system by showing that Müller-Eberhard's C3PA and properdin B are identical immunochemically.

This report concerns the activation of C3, C5, and C7 in the serum of a 9-yr old girl after incubation at 0°C for 6–8 h without utilization of C1, C4, and C2. The patient has upper respiratory infections associated with recurrent gross hematuria, focal glomerulonephritis, and transient renal insufficiency. The child was first noted to have gross hematuria at the age of 5 yr. At this time the hematuria was attributed to a urinary tract infection. A second episode of hematuria occurred when the patient was 8 yr of age and at this time a diagnosis of focal glomerulonephritis was made. In more recent months, the episodes of hematuria and infections have increased in frequency and at least a dozen attacks have occurred over the past year.

Three kidney biopsies were performed in 1971 (April, June, and November), B1c and fibrinogen were detectable to a varying degree along the glomerular basement membrane in a granular distribution. In a most recent study of the renal tissue (December 1972) properdin and IgA which were previously absent, could also be demonstrated. IgG, IgM, and C3PA were lacking in all tissues. It is the purpose of this report to detail our studies of the complement system and its activation in this patient. Evidence will be presented to indicate that the complement system of this patient is activated through C3 at 0°C. This activation requires the patient's IgA.

METHODS

Buffers for C assays. The disodium salt of ethylenediaminetetraacetic acid reagent grade Na₂H₂ EDTA was titrated to a pH 4 at a stock concentration of 0.15 M.

Na₂Mg₂EDTA (Geigy Chemical Corp., Ardsley, N. Y.) was also titrated to pH 4 at a stock concentration of 0.15 M. Gelatin veronal buffer and glucose gelatin veronal buffer with and without Ca⁺⁺ and Mg⁺⁺ (GGV⁺⁺, GGV⁻⁻) were prepared as described previously (3).

Serum. Blood was allowed to clot for 1 h at room temperature. The serum was removed after centrifugation at 4°C, aliquoted, and stored at -70°C until used.

Guinea pig C2. Partially purified C2 was prepared from guinea pig serum (Texas Biological Laboratory, Inc., Ft. Worth, Tex.) according to the method described by Nelson, Jensen, Gigli, and Tamura (4).

EAC1, EAC1A, and EAC4. Cell intermediates with C1 were prepared according to methods described previously (5, 6).

Assays of total complement (CH50) and the C components. Sensitation of erythrocytes from sheep (3) and the measurement of total complement in 50% hemolytic units (CH50) was carried out as described previously (7). Assays of human C1, C4, and C2 were determined according to published methods (5).

Assays of human C3, C5, C6, C7, C8, and C9. Functionally pure C components for the assays of these components and EAC1gp4-7th for use in assay of C8 and C9 were obtained from Cordis Laboratories (Miami, Fla.) and the assays were carried out according to the method described by Nelson et al. (4). The experimental error of the C components 1-9 ranged between 5 and 10%. C1q, C1s, and C3PA were carried out by the Mancini technique¹ (8).

Conversion of C3 was determined by immunoelectrophoresis using antisera against purified C3.

Conversion of C3PA was determined by immunoelectrophoresis according to the method of Götze and Müller-Eberhard (1) after treatment with inulin and with antisera against purified C3PA.²

Bactericidal activity was measured according to the method of Muschel and Treffers (9) using an *Escherichia coli* rough strain.

Phagocytic activity was measured according to the standard methods used in this laboratory adapted to using *Pseudomonas aeruginosa* type I (10).²

Method of isolation of active factor. 3 ml of the patient's plasma was applied to a 45 × 30 cm block of Pevikon C-870 (11) (Mercer Consolidated Corp., New York) prepared with barbital buffer, pH 8.6, ionic strength 0.05. The sample was applied at the cathodal region, electrophoresis was carried out at 350 V for approximately 18 h at 4°C. The blocks were cut into 14-cm segments and were eluted with barbital buffer. The eluates were analyzed for protein by Folin's method.

Assay of active fraction. Approximately 0.7 mg/ml of each fraction was reacted with 100 μl of normal serum for 2 h at 37°C. The mixture was serially diluted in GGV⁺⁺. 0.1 ml of EA1 × 10⁸/ml was then added to each tube and total hemolytic C was determined. The active factor appeared at the cathodal area and this fraction was concentrated in an Amicon ultrafiltration device (Amicon Corp., Lexington, Mass.) using the membrane filter UM-10. The concentrated fraction was analyzed by immunoelectrophoresis with monospecific antisera against IgA, IgM, and IgG.

¹ Antisera and Mancini plates were kindly supplied by Dr. Hans Müller-Eberhard.

² Biggar, W. D., N. K. Day, D. Windhorst, and R. A. Good. Impaired phagocytosis in C1r-deficient sera. Manuscript in preparation.

Purification by immunoadsorption. Monospecific anti-serum to IgA prepared according to methods described by Litman and Good (12) was coupled to activated, washed Sepharose according to previously described method (13). The antisera were shown to be specific for IgA by the methods outlined in the presentation of Litman and Good (12). 2 ml of plasma (70 mg/ml) was added to the Sepharose column. The fractions were eluted at a rate of 10 ml/h. Proteins were eluted over approximately 30 ml. Proteins eluted below OD 280 nm = 0.05 were not used for assay. The proteins bound to the immunoadsorbent were recovered at 2°C with 0.2 M glycine-HCl, pH 2.3.

RESULTS

Table I represents the total hemolytic complement (CH50) and each of the separate C components of fresh serum and serum after incubation at 0°C for 12 h. While total hemolytic C was elevated in the fresh serum (126 CH50 U/ml compared with the normal 80 CH50 U/ml), it was markedly reduced after incubation at 0°C (8-15 CH50 U/ml). This inactivation occurred after 8 h (Fig. 1). Normal serum upon comparable incubation showed no decrease in total complement. The decrease in total hemolytic C when the patient's serum was incubated at 37°C, however, occurred only gradually and only in some instances (Fig. 2) and not in others. Normal control serum remained essentially unchanged during this interval of incubation. The drop of total C in the patient's serum was associated mainly with a marked decrease of hemolytic C3 (9,500 CH50 U/ml - 33 CH50 U/ml) and to a variable extent of C5 and C7. Activation of the earlier components C1, C1q, C1s, C4, and C2 was not evident either by hemolytic (Table I) or immunochemical assays (Table II). Similarly, C8 and C9 were not reduced after incubation in the cold. The drop of hemolytic C3 was associated with conversion of C3 (Fig. 3) by immunoelectrophoretic analysis, and the formation of a diffuse ring when C3 was assayed by the Mancini technique (8). This conversion was unaffected when fresh serum was previously centrifuged at 20,000 rpm for 1 h suggesting that the activation was independent of any membranes or particulate matter that could have been present in the serum.

C3 proactivator as assayed by the Mancini technique in the fresh sample was reduced to 50% of the normal value (Table II). No further decrease in concentration was observed after incubation at 0°C. Although conversion of C3PA by inulin could be demonstrated in fresh or in serum kept at -70°C, conversion of C3PA by inulin was not demonstrable after the serum had been incubated in the cold (Figs. 4 and 5). This finding suggests that either maximal conversion of C3PA had occurred after incubation or that cofactors responsible for conversion of C3PA had been depleted by the incubation. When the patient's serum was de-

TABLE I
Total Hemolytic C and C Components in Fresh Serum and after Incubation at 0°C for 8 h in a Patient with Focal Glomerulonephritis

Patient	CH50	C1	C4	C2	C3	C5	C6	C7	C8	C9
Fresh	126	367,000	236,900	2,640	9,500	6,400	5,540	8,510	45,600*	9,697*
0°C	8-15‡	417,000	168,600	2,160§	33‡	3,500	5,600	4,940	47,880	11,515
Normal										
\bar{x}	80	430,000	246,000	1,350	2,600	3,415	6,150	5,529	65,889	15,795
1s	65-95	286,000- 574,000	144,500- 347,000	850- 1,850	2,068- 3,132	2,595- 4,235	5,059- 7,241	4,094- 6,964	46,311- 85,467	9,946- 20,644
2s	50-110	145,000- 721,000	43,000- 449,000	350- 2,350	1,536- 3,664	1,775- 5,055	3,968- 8,332	2,659- 8,399	26,733- 105,045	4,097- 27,493

\bar{x} , Mean values of 40 healthy adults.

1s, values representing first standard deviation.

2s, values representing second standard deviation.

* Below first standard deviation.

‡ Below second standard deviation.

§ Above first standard deviation.

|| Above second standard deviation.

pleted of C3PA by adsorption through a Sepharose column containing specific anti-C3PA and incubated overnight at 0°C, the activation of C was reduced by 50% (Table III) and C3 was not converted. This would suggest that C3PA is utilized in the activation

of the patients serum. Properdin activity measured by hemolytic assay according to a modified method of Pillemer et al. was also decreased after incubation (14, 15).

Requirement of Mg⁺⁺ for activation at 0°C. When

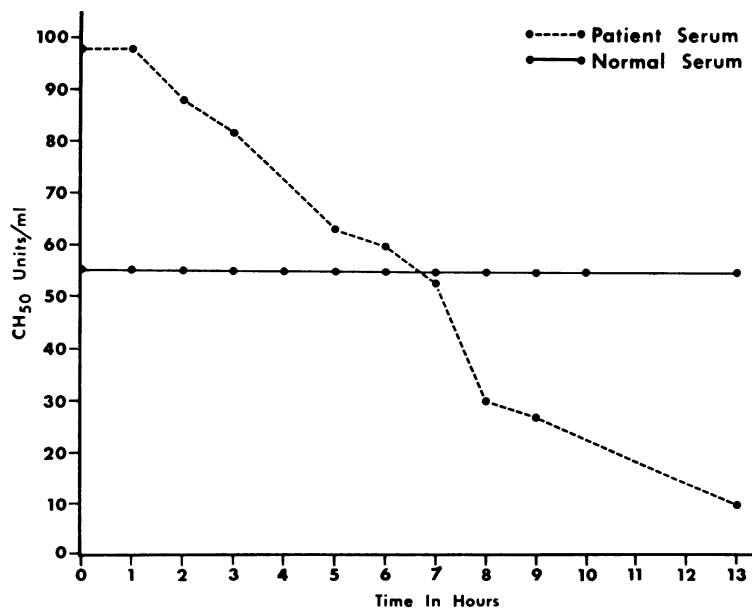


FIGURE 1 Kinetic study of patient's serum at 0°C with recurrent hematuria and focal glomerulonephritis. Patient's serum was compared with normal serum at 0°C for 13 h. Sera were assayed for total hemolytic complement activity at varying intervals of time. Total hemolytic C of the patient's serum dropped markedly after 8 h. Normal control serum remained unchanged.

TABLE II
*Immunochemical Assay of C Components in Fresh Serum and after Incubation at 0°C for 8 h in a Patient with Focal Glomerulonephritis**

Patient	C1q	C1s	C4	C3‡	C5	C6	C8	C9	C3PA§
Fresh	232	174	610	354	89	206	31	260	118
0°C	207	162	670	390	84	180	27	216	121
Normal	208	138	402	990	77	116	54	150	237

* Mancini plates containing antisera to the above components were kindly supplied by Dr. Hans Müller-Eberhard. Results are expressed as milligrams N per milliliter.

‡ The increase in C3 was due to conversion of C3 (See Fig. 3).

§ C3 proactivator.

blood was drawn from the patient in acid citrate dextrose (ACD) solution, the complement system was not activated by incubation at 0°C. Activation was only evident after the addition of Mg⁺⁺ ions. Fig. 6 represents the dose-response curve obtained after the addition of varying amounts of Mg⁺⁺ to the patient's plasma and incubation at 0°C overnight. As demonstrated, whereas normal control serum indicated a rise in total hemolytic complement at the optimum concentration of Mg⁺⁺ and the patient's total hemolytic C dropped dramatically at

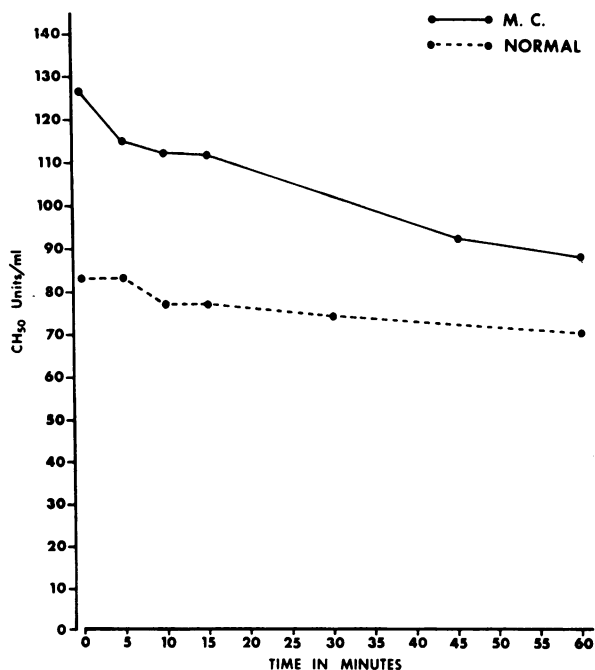


FIGURE 2 Kinetic study of patient's serum at 37°C with recurrent hematuria and focal glomerulonephritis. Patient's serum was compared with normal serum at 37°C for 1 h. Sera were assayed for total hemolytic complement at varying intervals of time. M. C. denotes patient. There was a gradual drop of total hemolytic C in the patient's serum when compared with normal serum.

this concentration of Mg⁺⁺ indicating that the activation at 0°C required Mg⁺⁺ ions. No change in total hemolytic C was observed when Ca⁺⁺ ions were substituted for Mg⁺⁺ in the above experiment.

Bactericidal and phagocytic activity. Studies of the bactericidal activity of the patient's serum before and after incubation at 0°C are presented in Table IV. As indicated, a 40-fold increase in the volume of the patient's serum was required to exhibit bactericidal activity after incubation at 0°C as compared with fresh unincubated serum. Similarly, evidence that a phagocytic defect for *P. aeruginosa* appeared upon incubation at 0°C is presented in Fig. 7.

Isolation of active factor by Pevikon block electrophoresis. When the normal serum was incubated with 17 plasma fractions from Pevikon block at 0°C overnight and then assayed for total hemolytic complement by the microtiter method, it was found that the third fraction was most anticomplementary. The experiment was set up again as above, using three fractions, fractions 2, 3, and 17 and the total hemolytic complement CH₅₀ was determined by macrotiter. Table V represents the results obtained. A reduction of about 88% of

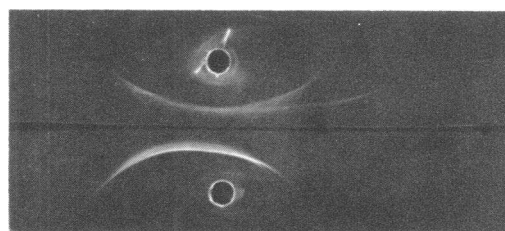


FIGURE 3 Immunoelectrophoretic pattern of patient's serum with focal glomerulonephritis using 2% agar, barbital buffer pH 8.5 ionic strength 0.05 containing 0.01 M EDTA and a potential gradient at 6 V/cm for 2½ h at 10°C. Middle trough contains antiserum against purified C3. Upper well which demonstrates conversion of C3 contains patient's serum after incubation at 0°C for 12 h. Lower well contains normal serum incubated under the same conditions as the patient's serum.

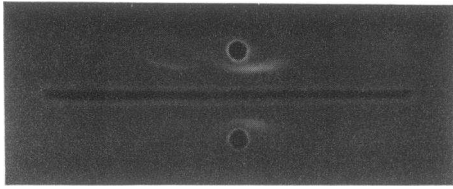


FIGURE 4 Immunoelectrophoretic pattern of patient's serum after treatment with inulin according to the method of Götze and Müller-Eberhard (1). 0.1 ml of the patient's freshly frozen serum (lower well) and normal human serum also freshly frozen (upper well) was incubated with 20 μ l of a 10 mg/ml inulin suspension. The conditions for electrophoresis are described in Fig. 3. Conversion of C3 proactivator occurs in both the patient's serum and in normal serum.

CH50 with fraction 3 occurred as compared with no reduction produced by fraction 17. Some inhibition was also obtained with fraction 2. The three fractions were then concentrated separately in an Amicon ultrafiltration device (Amicon Corp.) using the membrane filter UM-10. The active fractions were concentrated and were analyzed by immunoelectrophoresis and by Ouchterlony with antisera against IgG, IgM, and IgA. A band appeared with antisera against IgA in fraction 3 which was the most active fraction.

Purification by immunoadsorption. When the patient's plasma which had been adsorbed by a Sepharose column containing antiserum specific for IgA was incubated with Mg^{++} (2 mg/ml) overnight at 0°C, the CH50 activity dropped only by 4% as compared with unadsorbed control patient's plasma which was incubated with Mg^{++} at 0°C overnight. The C activity in the control plasma dropped by greater than 95% (Table VI).

Similarly, when the above specific IgA adsorption was repeated with the patient's serum and the adsorbed serum then incubated overnight at 0°C, the drop in hemolytic C was only 20% and no conversion of C3 could be demonstrated (Fig. 8). When IgM was specifically removed from the patient's serum by immunoadsorption, the percent inhibition of complement was

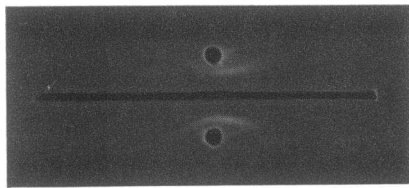


FIGURE 5 Immunoelectrophoretic pattern of patient's serum (lower well) and normal serum (upper well) after incubation at 0°C for 12 h. Middle trough contains antiserum to C3PA. The conditions of immunoelectrophoresis were identical as described in Fig. 4. No conversion of C3PA is demonstrable in the patient's serum.

TABLE III

Percent Inhibition of Total Hemolytic Complement CH50 and Conversion of C3 of Patient's Serum Depleted of C3PA and after Incubation at 0°C Overnight

	CH50	% Inhibition	C3 conversion
Serum control	90	>90	Yes
Serum depleted of C3PA	66	50	No

61% (Table VII). These experiments indicated that IgA is responsible for activation of the C system in this patient's serum. In order to determine whether the Sepharose alone was absorbing nonspecifically one or more proteins involved in the activation of C3 in the cold, patient's plasma was applied to a Sepharose column not charged with any antiserum. The fractions containing the highest activity of hemolytic C were left overnight at 0°C with Mg^{++} . As shown in Table

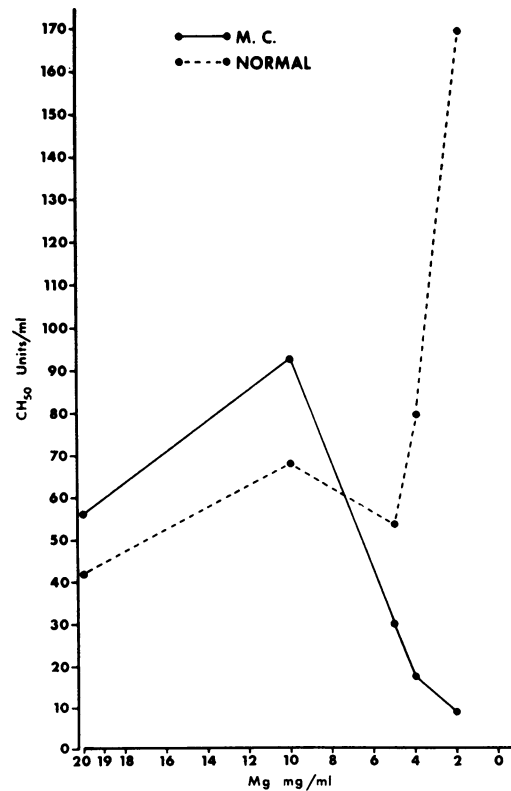


FIGURE 6 Effect of Mg^{++} ions on patient's plasma and incubation at 0°C for 12 h. As demonstrated inhibition of total complement occurred in the patient's plasma at the concentration necessary for maximum lysis. In contrast, control plasma showed maximum lysis at the same concentration. M. C. represents patient's plasma.

TABLE IV
Bactericidal Activity of Fresh Serum and after Incubation at 0°C for 8 h in a Patient with Focal Glomerulonephritis

	Bactericidal activity*	
	<i>E. coli</i> ‡	
	Fresh	Incubated
Patient	0.009	0.035
Normal	0.0074	0.0065

* Bactericidal activity performed according to the method of Muschel and Treffers (9). Results are expressed as the amount of serum necessary to kill 50% bacteria.

‡ *E. coli* rough strain.

VIII, the CH50 activity dropped by 80%. The activation of the control patient's serum was 94%. The above immunoadsorption experiments were performed on the same day and the columns used were identical in size. The hemolytic assays were carried out the following day. In addition, when the fractions passed over such a column were analyzed by immunoelectrophoresis with

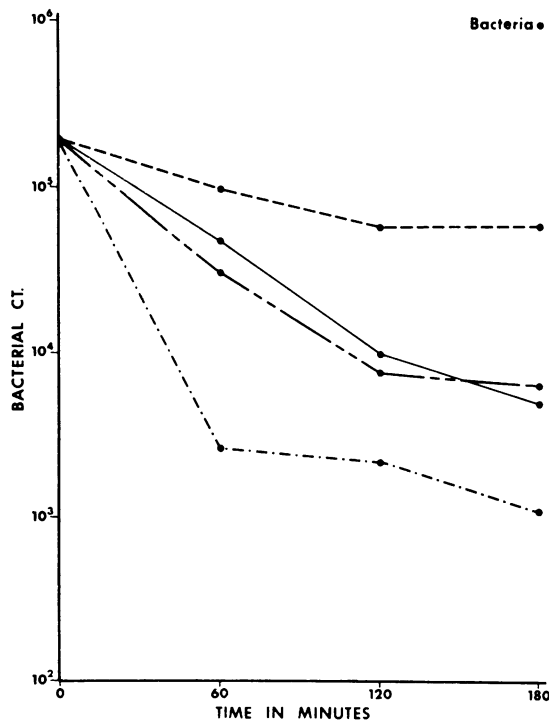


FIGURE 7 Defect of phagocytic function of the patient's serum after incubation at 0°C. (· · · · ·), Patient's serum after incubation at 0°C for 8 h. (· — · — ·), Pool normal human serum after incubation at 0°C for 8 h. (· — · — ·), Pool normal human serum. (· · · · ·), Patient's serum at -70°. The patient's serum was defective in the phagocytic function for *P. aeruginosa* type I after incubation at 0°C.

TABLE V
Anticomplementary Activity of Patient's Plasma Fractions Obtained by Electrophoresis on Pevikon Block on Normal Human Serum

Fraction no.	Percent inhibition CH50 U/ml
2	45
3	88
17	<5

3 ml of the patient's plasma was applied to a 45 cm × 30 cm block of Pevikon prepared with barbital buffer, pH 8.6, ionic strength 0.05. The sample was applied at the cathodal region. Electrophoresis was carried out at 350 V for approximately 18 h at 4°C. The blocks were cut into 1¼-cm segments and were eluted with barbital buffer. The eluates were analyzed for protein by Folin's method.

Approximately 0.7 mg/ml of each fraction was reacted with 100 µl of normal serum for 2 h at 37°C. The mixture was diluted in GG^{V++}. 0.1 ml of EA1 × 10⁸/ml was then added to each tube and total hemolytic C was determined. The results represent percent inhibition of total CH50 when compared with the control fraction—fraction 17.

antisera against IgG, IgM, IgA, and IgD, none of the immunoglobulins were removed nor was C3PA removed.

A control experiment to determine whether IgG was involved in the activation of C3 was then executed. Since it was difficult to remove IgG completely from the patient's serum by a single immunoadsorption and since subsequent adsorptions led to a high dilution of the serum, the following experiment was carried out.

The patient's plasma was applied to a Pevikon block and those fractions containing a mixture of IgG and IgA were added to normal serum and incubated at 0°C overnight. Inhibition of CH50 activity in the normal serum occurred. The IgA from these fractions was removed on Sepharose column containing anti-IgA. The purity of these adsorbed fractions was established by immunoelectrophoresis and Ouchterlony (Fig. 9). These fractions were then added to normal human

TABLE VI
Percent Inhibition of Total Hemolytic Complement CH50 and Conversion of C3 of Patient's Fresh Plasma, of Plasma Depleted of IgA and after Incubation at 0°C Overnight

	CH50	% Inhibition	C3 conversion
Plasma control	130	>95	yes
Plasma depleted of IgA	61	4	no

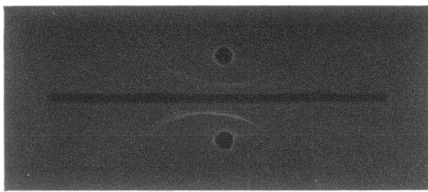


FIGURE 8 Immunoelectrophoretic pattern of patient's serum with focal glomerulonephritis after immunoadsorption on IgA Sepharose column. Upper well contains patient's serum after incubation at 0°C for 12 h. Lower well contains patient's serum incubated under the same conditions but after immunoadsorption through a Sepharose column containing antiserum against IgA. No conversion of C3 is demonstrable after removal of IgA.

serum and incubated overnight at 0°C, as presented in Table IX. The serum control incubated overnight with buffer showed no drop in total hemolytic C and no conversion of C3. Mixtures of serum with the IgG fractions, with the IgA fractions and with both IgG and IgA fractions were incubated overnight at 0°C. With the IgG fraction only slight inhibition of CH50 (7%) occurred and there was no conversion of C3. With patient's IgA, the inhibition was greater than 90% and conversion of C3 was observed. With the mixture of IgG and IgA, the inhibition of CH50 activity was 89% and conversion of C3 could be demonstrated.

When IgA fractions from normal serum were prepared in the same way, little or no activation of the C3 pathway occurred after incubation with normal serum in the cold overnight. When the purified IgA removed from the patient's serum was added back to the serum depleted of IgA, the capacity to deplete the complement activity in the cold was restored.

These experiments indicate that IgA is responsible for the activation of the C system in this patient's serum and suggest that the patient's IgA is either abnormal or is reacting with a component in normal serum which activates C3 in the cold.

DISCUSSION

Our observations illustrate an unusual mode of activation of the complement system in vitro in a patient with

TABLE VII
Percent Inhibition of Total Hemolytic Complement of Plasma Depleted of IgM and after Incubation at 0°C Overnight

	CH50	% Inhibition
Plasma control	130	>95
Plasma depleted of IgM	62	61

TABLE VIII
Percent Inhibition of Total Hemolytic Complement CH50 of Patients Fresh Serum, of Patient's Serum after Passage through Plain Sepharose Column and after Incubation at 0°C Overnight

	CH50	% Inhibition
Serum control	130	94
Serum fraction after passage through Sepharose	60	80

progressive renal disease. The activation is most marked at 0°C and is associated with a dramatic drop of hemolytic C3 and conversion of C3. In addition, the opsonic effect of the patient's serum to *Pseudomonas* organisms and the serum bactericidal activity is also reduced upon incubation of the patient's serum in the cold. The activation in the cold seems to require the patient's IgA.

Histological lesions demonstrate the presence of B1c, IgA, and properdin. These data suggest that the activation of the complement system in the patient's serum which is more evident at 0°C, is probably a continuous process occurring in vivo and thus very likely associated with the pathogenesis of the patient's recurring disease.

Prior studies in this laboratory and in others linking isolated deficiencies of the C system with increased frequency of renal disease, vascular disease, and susceptibility to infection (16-22) make it necessary to consider the possibility that the frequent infections in our patient may relate to the abnormality of the complement system. The bactericidal and opsonic defects might argue for this possibility. On the other hand, it seems entirely possible that the infections or their consequences, e.g., some form of autoimmunity, might underlie the activation of the complement system in this particular manner.

Whatever is actually activating the patient's serum at 0°C requires Mg⁺⁺ not Ca⁺⁺. From studies of other investigators, Mg⁺⁺ is required for the alternate proactivator pathway and the function of the properdin system (1, 2, 14, 23).

In the present studies, the level of the proactivator as determined by the Mancini technique is reduced in the patient's serum even before activation in the cold by some 50% as is the total properdin level as measured by functional assays. In addition, when the patient's serum is depleted of C3PA by specific immunoadsorption, the activation is reduced by 40-50%. Further, upon incubation of the patient's serum with inulin after incubation at 0°C, no conversion of C3PA is detectable. These data suggest that C3PA or component(s) responsible for the inulin activation of C3PA, e.g., C3PA

convertase are utilized during the spontaneous activation of C3PA in vitro.

Although C3PA is not demonstrable in the kidney biopsies by immunofluorescent techniques, IgA, and properdin are present. Direct evidence that this patient has continuous activation in vivo of the C3PA as well is not yet available and will require studies of utilization of these components which have not yet been carried out. Recent studies by Götze and Müller-Eberhard (1) and by Goodkofsky and Lepow suggest that C3PA and properdin B are identical (2). In our studies we have not measured properdin A, but it might be desirable to make such studies in light of our observations.

The conversion of C3 at 0°C is indeed a provocative finding. Whether an as yet unknown enzyme is responsible for this activation or whether the patient has an activator such as an antibody or autoantibody in her serum which operates preferentially by the alternate pathway in the cold must be studied further. Already, however, it is clear that IgA is required for this strange form of complement activation. Evidence from Pevikon block fractionation indicates that the responsible serum component is patient's IgA. When IgA is removed from either the patient's plasma or serum by immunoadsorption and then the serum is kept overnight at 0°C, no conversion of C3 or drop in hemolytic titer occurs. When IgA obtained from Pevikon block fractions of patient's plasma is added to the patient's serum depleted of IgA, a drop in hemolytic complement and a conversion of C3 occurs in the cold. When IgG or IgA or mixtures of IgG and IgA obtained by Pevikon block fractionation from the patient's serum are added to normal serum, and the mixture kept overnight at 0°C, the hemolytic titer of serum drops by greater than 80% and a conversion of C3 occurs within those mixtures containing IgA, whereas with IgG alone, there is less than 10% drop in hemolytic titer and no conversion of C3. Application of the patient's serum on Sepharose without antiserum or with Sepharose activated with IgM, does not remove nonspecifically any of the proteins required for the activation of

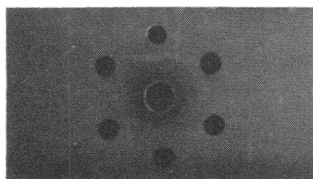


FIGURE 9 Ouchterlony pattern of patients plasma after Pevikon block fractionation and after passage through sepharose column containing anti-IgG antiserum. Upper well contains fraction after absorption of IgG absorption. Center well contains anti-IgA antiserum.

TABLE IX
Percent Inhibition of Total Hemolytic Complement CH50 and Conversion of C3 after Incubation at 0°C of Normal Serum with Pevikon Block Fractions of Patient's Plasma Containing IgG, IgA, and a Mixture of IgG plus IgA

	CH50	% Inhibition	C3 conversion
Serum control	110	<5.0	No
Serum + IgG	102	7.3	No
Serum + (IgG + IgA) Mixture	<10	>90.0	Yes
Serum + IgA	12	89.0	Yes

the C system in the cold from either the patient's or normal sera.

Although present understanding of the complement system, the so-called alternate pathway, and the relationships of these to the properdin system are not yet sufficiently definitive to permit us to explain completely the association of recurrent renal disease with the unusual cold activation of the complement system that we have observed in the serum of our patient, it seems certain that these phenomena are intimately related in an important way. To our knowledge, this patient's serum is unique in that its IgA activates complement in the cold without utilizing C1, C4, and C2. The association of this unique serological process with the clinical picture of recurrent hematuria in unexplained renal disease makes it necessary to look at other patients who have recurrent unexplained hematuria for similar serological perturbations and to look more regularly especially in patients with renal disease and recurrent infection for evidence of spontaneous activations of the alternate pathway by the methodology that was used in the study of this patient. Since the discovery of cold activation of the complement system was accidental in this instance, it seems likely that directed search for this activation mechanism will reveal other cases that can shed light on a most provocative association.

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REFERENCES

1. Götze, O., and H. J. Müller-Eberhard. 1971. The C3-activator system: an alternate pathway of complement activation. *J. Exp. Med.* **134** (Suppl.): 905.
2. Goodkofsky, I., and I. H. Lepow. 1971. Functional relationship of factor B in the properdin system to C3 proactivator of human serum. *J. Immunol.* **107**: 1200.
3. Mayer, M. M. 1961. Procedure for titration of complement components with R₁, R₂, R₃ and R₄. In *Experimental Immunochemistry*. E. A. Kabat and M. M. Mayer, editors. Charles C Thomas, Publisher, Springfield, Ill. 2nd edition. 163.
4. Nelson, R. A., Jr., J. Jensen, I. Gigli, and N. Tamura. 1966. Methods for the separation, purification and measurement of nine components of hemolytic complement in guinea pig serum. *Immunochemistry*. **3**: 111.
5. Gewurz, H., A. R. Page, R. J. Pickering, and R. A. Good. 1967. Complement activity and inflammatory neutrophil exudation in man: studies in patients with glomerulonephritis, essential hypocomplementemia and agammaglobulinemia. *Int. Arch. Allergy Appl. Immunol.* **32**: 64.
6. Borsos, T., and H. Rapp. 1967. Immune hemolysis: a simplified method for the preparation of EAC4 with guinea pig or with human complement. *J. Immunol.* **99**: 263.
7. Day, N. K., R. J. Pickering, H. Gewurz, and R. A. Good. 1969. Ontogenetic development of the complement system. *Immunology*. **16**: 319.
8. Mancini, G., A. O. Carbonara, and J. F. Heremans. 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*. **2**: 235.
9. Muschel, L. M., and H. B. Treffers. 1956. Quantitative studies on the bactericidal actions of serum and of complement. I. A rapid photometric growth assay for bactericidal activity. *J. Immunol.* **76**: 1.
10. Maaløe, O. 1946. Doctoral Thesis. Munksgaard, Copenhagen.
11. Müller-Eberhard, H. J. 1960. A new supporting medium for preparative electrophoresis. *Scand. J. Clin. Lab. Invest.* **12**: 33.
12. Litman, G. W., and R. A. Good. 1972. Rapid purification of IgA from normal human serum. *Biochim. Biophys. Acta.* **263**: 89.
13. Frommel, D., J. M. Dupuy, G. W. Litman, and R. A. Good. 1970. Use of immunoadsorbent techniques in the preparation of chemical agammaglobulinemia. *J. Immunol.* **105**: 1292.
14. Pillemer, L., L. Blum, I. H. Lepow, O. A. Ross, E. W. Todd, and A. C. Wardlaw. 1954. The properdin system and immunity. I. Demonstration and isolation of a new serum protein, properdin and its role in immune phenomenon. *Science (Wash. D. C.)*. **120**: 279.
15. McLean, R. H., and A. F. Michael. 1972. The immunoelectrophoretic pattern of properdin in fresh and aged human serum. *Proc. Soc. Exp. Biol. Med.* **14**: 403.
16. Kohler, P. F., and H. J. Müller-Eberhard. 1969. Complement-immunoglobulin relation: deficiency of C1q associated with impaired immunoglobulin synthesis. *Science (Wash. D. C.)*. **163**: 474.
17. Gewurz, H., R. J. Pickering, and R. A. Good. 1968. Complement and complement component activities in diseases associated with repeated infections and malignancy. *Int. Arch. Allergy Appl. Immunol.* **33**: 368.
18. Pickering, R. J., G. B. Naff, R. M. Stroud, R. A. Good, and H. Gewurz. 1970. Deficiency of C1r in human serum. Effects of the structure and function of macromolecular C1. *J. Exp. Med.* **131**: 803.
19. Moncada, B., N. K. B. Day, R. A. Good, and D. Windhorst. 1972. Lupus-erythematosus-like syndrome with a familial defect of complement. *N. Engl. J. Med.* **286**: 689.
20. Day, N. K., H. Geiger, R. Stroud, M. de Bracco, B. Moncada, D. Windhorst, and R. A. Good. 1972. C1r deficiency—an inborn error associated with clinical and renal disease. *J. Clin. Invest.* **51**: 1102.
21. Agnello, V., M. M. E. deBracco, and H. G. Kunkel. 1972. Hereditary C2 deficiency with some manifestations of systemic lupus erythematosus. *J. Immunol.* **108**: 837.
22. Day, N. K., H. Geiger, R. McLean, A. Michael, and R. A. Good. 1973. C2 deficiency. Development of lupus erythematosus. *J. Clin. Invest.* **52**: 1601.
23. Lepow, H. J. 1972. Biologically active fragments of complement. In *Progress in Immunology*. Bernard Amos, editor. Academic Press, Inc., New York. 589.