

Hereditary deficiency of the second component of complement (C'2) in man.

M R Klemperer, ... , F S Rosen, K F Austen

J Clin Invest. 1966;[45\(6\)](#):880-890. <https://doi.org/10.1172/JCI105403>.

Research Article

Find the latest version:

<https://jci.me/105403/pdf>



Hereditary Deficiency of the Second Component of Complement (C₂) in Man*

MARTIN R. KLEMPERER,† HAROLD C. WOODWORTH, FRED S. ROSEN,‡ AND
K. FRANK AUSTEN §

(From the Department of Medicine, Children's Hospital Medical Center, Boston, Mass.; the Communicable Disease Center of the U. S. Public Health Service, Atlanta, Ga.; the Department of Medicine, Massachusetts General Hospital; and the Departments of Pediatrics and Medicine, Harvard Medical School, Boston, Mass.)

The serum complement system is composed of at least eight or nine interacting proteins (1, 2). The complete system is required for the reactions of immune cytotoxicity, immune hemolysis, bacteriolysis, and the lysis of certain tumor cells (1, 3-7). There are other complement dependent systems that do not require all nine components. The reactions of immune adherence and erythrophagocytosis require the first four reacting components of the complement system (2, 8-10). The generation of a chemotactic factor for polymorphonuclear leukocytes requires the activity of the first six reacting complement components (11, 12). Knowledge of the involvement of the serum complement system in these noncytolytic reactions has been acquired only recently and has greatly broadened our concepts of the possible role of the complement system in normal and morbid processes.

In addition to these experimental findings, the occurrence of a lowered serum complement level in various acquired pathological states has been well documented (13-17), and the precise cause of the decreased hemolytic activity has been established in at least one human inborn abnormality, hereditary angioneurotic edema (HANE) (18).

* Submitted for publication December 20, 1965; accepted February 24, 1966.

Presented in part at the 38th Annual Meeting of the Central Society for Clinical Research, Chicago, Ill., November 6, 1965.

Supported by grants AI-AM-05877-02, GM-09107-05, and AI-04536-05.

† Address requests for reprints to Dr. Martin R. Klemperer, Children's Hospital Medical Center, 300 Longwood Ave., Boston 15, Mass.

‡ Recipient of U. S. Public Health Service Career Development Award (1-K3-AM-19650-02).

§ Recipient of U. S. Public Health Service Career Development Award (5-K3-AI-9702-05).

Among mammalian species specific inborn deficiencies involving a single complement component have been defined in rabbits (19) and mice (20), and a deficiency within the classical third component complex has been established in guinea pigs (21). The present paper defines an inborn deficiency of the second component of human complement. Affected individuals have been identified in each of the three generations of a kindred.

Methods

Serum samples were separated from freshly clotted blood specimens that had been allowed to stand at room temperature for 30 minutes and then centrifuged at 0 to 4° C. All sera were then either immediately stored as small portions at -65° C or, if transported, frozen and kept in dry ice. The transported sera were then thawed, quickly subdivided into small portions, and stored at -65° C. A serum sample was thawed once for determinations involving the complement system.

Determination of the serum hemolytic complement titer (C_{H50}). The method used was a 3-ml modification (22) of the 7.5-ml assay of Kabat and Mayer (23). Consequently, all reagents used in the titration were decreased in volume by 60%. This method yielded results comparable to the 7.5-ml volume titration (24). Sheep erythrocytes¹ and hemolysin² were obtained, and the same lot of hemolysin was used for all hemolytic determinations. Saline-barbital buffer, pH 7.3, with 0.1% wt/vol crystallized bovine plasma albumin³ and the sensitized sheep erythrocyte suspension have been described (23). The complement titer was obtained by plotting the log (y/1-y), where y equals the per cent of lysis, against the volume of diluted serum on 2- \times 3-cycle log log graph paper. When sera at low dilutions were used in the titrations, serum blanks were included, and the optical density of these was subtracted from that of the lysates in tubes containing serum as a correction for serum color.

¹ Preserved sheep cells, Hyland Laboratories, Los Angeles, Calif.

² Sheep cell hemolysin from Hyland Laboratories.

³ Armour Pharmaceutical Company, Kankakee, Ill.

Titration of the serum complement components. Reagents (R1, R2, R3, R4) each deficient in one of the four classical complement components were prepared from fresh human serum and stored at -65°C . The volume of reagent used in the titration of each complement component yielded complete lysis in recombination with each of the other three reagents and by itself lysed less than 5% of the sensitized sheep cell suspension used in the assay. Titrations were performed by the method described by Wedgwood (25). All titrations were performed simultaneously with a known normal serum control.

The second component of complement of human serum was also assayed with the stoichiometric titration of Austen and Beer (26), employing sensitized sheep erythrocytes persensitized with guinea pig first and fourth complement components (EAC'la^{sp},4^{sp}). The possibility that the C'2^{hu} titer, as measured by the interaction of whole serum and EAC'la^{sp},4^{sp} cells, requires the participation of human fourth component of complement (C'4^{hu}) was suggested by Nelson (2) and has recently been confirmed (27). Apparently C'la^{sp}, transferred from SAC'la^{sp},4^{sp} sites, reacts with C'4^{hu} to form SAC'la^{sp},4^{hu} sites early in the t_{\max} period; the stoichiometric titration of C'2^{hu} is then due primarily to the interaction of this component with the newly formed SAC'la^{sp},4^{hu} sites rather than with SAC'la^{sp},4^{sp} sites. Since the source of C'4 cannot be designated with absolute certainty, the sites formed by the interaction of whole human serum with EAC'la^{sp},4^{sp} cells have been designated SAC'la^{sp},4*,2^{hu} in Results. Sheep erythrocytes and hemolysin for this assay were obtained commercially.⁴ Second component titrations were performed with a reference normal serum of known C'2 content, and all values were corrected to an assigned base value for the reference serum (28). The R2 used for the second component titrations contained 28 C'2 U per ml when assayed with an EAC'la^{sp},4^{sp} cell titration.

Equal amounts of standardized EAC'la^{sp},4^{sp} cells were mixed with a 1:500 dilution of normal human serum or with a 1:10 dilution of C'2 deficient human serum to determine the incubation time at which the greatest number of sites on the erythrocyte surface are converted from SAC'la^{sp},4^{sp} to SAC'la^{sp},4*,2^{hu}. This optimal incubation time is termed the t_{\max} time.

The effect of adding highly purified C'2 to C'2 deficient serum. Highly purified human C'2 (29) ⁵ in buffer containing 1×10^{-3} M EDTA and 1×10^{-4} M dithioerythritol represented an approximate 30-fold concentration of the serum C'2 content. We added 0.04 ml of the purified human C'2 to 1 ml of C'2 deficient serum, and we measured hemolytic serum complement activity and the C'2 titer in the reconstituted serum with the methods described above.

Functionally pure guinea pig C'2 ⁶ (30) was added in in-

creasing amounts of up to 120 U per ml to a 1:40 dilution of C'2 deficient human serum. The mixture volume was kept constant at 0.4 ml. Standardized sensitized sheep erythrocytes (23), 0.4 ml, and barbital buffer were added to make a total volume of 3 ml. The per cent of lysis was determined for each increment of functionally pure guinea pig C'2 added and then plotted against units of guinea pig C'2 added on arithmetic graph paper. The amount of functionally pure guinea pig C'2 required for 50% lysis was then obtained by plot.

Immunodiffusion studies of the serum beta_{1c} (C'3)-, beta_{1E} (C'4)-, and beta_{1F} (C'5)-globulins and C'1 esterase inhibitor. Immuno-electrophoresis was performed with the method of Scheidegger (31). Rabbit anti-beta_{1c}-beta_{1E}-globulin ⁷ and goat anti-beta_{1c}-globulin ⁸ were obtained commercially. The latter serum also gave a precipitation band with beta_{1F}-globulin. Rabbit anti-beta_{1c}-globulin was prepared by injecting rabbits with pure human beta_{1c}-globulin isolated by the method of Müller-Eberhard, Nilsson, and Aronsson (32). Rabbit antiserum to C'1 esterase inhibitor was prepared by injecting rabbits with purified C'1 esterase inhibitor (E-I).⁹ All patterns were developed at room temperature and stained with amido black.

The serum beta_{1c}-globulin was determined by quantitative immunodiffusion, as described by Klemperer and his co-workers (34). Conversion of serum beta_{1c}-globulin was assessed by adding washed rabbit antihuman gamma globulin-gamma globulin precipitates to normal and to C'2 deficient human sera. The mixtures were incubated in a 37° water bath and samples removed at 20-minute intervals. The further conversion of beta_{1c}-globulin was prevented by delivering the samples into buffered pH 7.4 EDTA at 0° C. Sera incubated without the addition of washed immune precipitates were used as controls. The conversion of serum beta_{1c}-globulin was estimated by use of immunoelectrophoresis. A similar experiment was performed in which the washed immune precipitates were added to sera containing buffered EDTA at 0.01 M final concentration.

The serum concentration of C'1 esterase inhibitor was determined by immunodiffusion with the technique described by Rosen, Charache, Pensky, and Donaldson (35). Functional serum C'1 esterase inhibitor activity was measured by the method of Levy and Lepow (36).

Determination of the serum bactericidal capacity. The bactericidal capacity of the sera tested was determined by the method of Michael, Whitby, and Landy (37), using *Salmonella typhosa* 0901. Bactericidal assays were performed using unabsorbed serum. In all assays 0.1 ml of immune rabbit anti-*S. typhosa* 0901 serum was added to the system, so that the reactions were carried out with serum complement as the limiting factor. In the assay the end point was interpreted as the serum dilution

⁴ Baltimore Biological Laboratories Division, BD Laboratories, Inc., Baltimore, Md.

⁵ Generously supplied by Dr. Irwin H. Lepow.

⁶ Generously donated by Dr. Manfred M. Mayer.

⁷ Anticomplement (β_{1c}/β_{1E} -globulin) (Behringwerke), obtained from Diagnostic Reagents Division of Lloyd Brothers, Inc., Cincinnati, Ohio.

⁸ Hyland Laboratories.

⁹ Prepared by Dr. Jack Pensky (33).

at which 50% of the organisms by colony count were killed. C'2 deficient human serum reconstituted with highly purified human C'2 was also tested for its bactericidal activity. C'2 deficient serum was run in parallel with the same serum reconstituted.

Determination of the serum complement titer by immune adherence (C'I-A₅₀). The immune-adherence titer was determined by a modification of the method of Woodworth (38) using a *Brucella abortus*-anti-*Br. abortus* system (39). *Br. abortus*¹⁰ was washed twice in barbital buffer by centrifuging at $6,600 \times g$ for 15 minutes. Anti-*Br. abortus* was obtained commercially.¹¹ The optimal dilutions of antibody and antigen were determined by two-dimensional titration of anti-*Br. abortus* 1:160 vs. *Br. abortus* suspension giving an optical density of 0.150 at 650 m μ measured with a Beckman DU spectrophotometer. The test system was composed of 0.2 ml antigen, 0.2 ml antibody, 0.5 ml human serum dilutions, and 0.1 human type "O" erythrocytes that had been standardized so that the optical density of a 1:10 lysate equaled 0.395 at 541 m μ in a Beckman DU spectrophotometer. This erythrocyte suspension contained approximately 2.6×10^7 cells per ml. All reagents were made up with saline-barbital buffer containing bovine plasma albumin. Adherence of *Br. abortus* to human erythrocytes was determined by counting 100 organisms under phase contrast at $900 \times$ magnification. The number of organisms adherent to the human erythrocytes equaled the per cent adherence at a given serum dilution. All determinations were made in duplicate, and the average per cent adherence vs. serum dilution was plotted on semilog paper. The serum dilution at which 30% of the organisms were adherent to the erythrocytes was obtained by plot. This was designated the C'I-A₃₀. A reference serum with an assigned C'I-A₃₀ of 1,280 was included with all I-A determinations, and all values were adjusted to this reference serum as a standard. The effect of reconstituting C'2 deficient human serum with highly purified human C'2 was also determined by using this method. C'2 deficient serum was run in parallel with the same serum reconstituted.

Medical histories of C'2 deficient individuals. The propositus (III, 7) is a 45-year-old male. He had measles, mumps, chicken pox, and scarlet fever during childhood without any untoward complications or sequelae. He has never had any undue susceptibility to infection. At 40 he had acute maxillary sinusitis treated by irrigation of the sinus with penicillin. Nine days later he developed a severe serum sickness type of penicillin reaction with redness, swelling and pain in both ankles, arthralgia of the elbows and wrists, an edematous vesicular eruption of the feet, and urticaria of the trunk. The symptoms were ameliorated by triamcinolone. He has had poison ivy once. At 25 he was found to be tuberculin-positive, and at 37 he gave a positive response to first

strength purified protein derivative. The deficiency was discovered by the propositus while performing studies on immune adherence with his own serum.

A sister of the propositus (III, 10) is 43 years old. She had uncomplicated varicella, rubella, and mumps during childhood. At age 1½ she had bilateral pneumonia with empyema. She was again hospitalized for pneumonia at 24. At 34 she had an episode of pleurisy, considered to be of viral etiology. During the same year a hand infection complicated by lymphadenitis was successfully treated with antibiotics. She had otitis media at ages 6, 8, and 21 years. She has never had an untoward reaction to penicillin. One episode of mild poison ivy was sustained in childhood. The tuberculin reaction was negative when last tested 20 years ago.

A brother of the propositus (III, 11) is 40 years old. He had measles, chicken pox, and mumps without complication during childhood. He had two episodes of otitis media as a child. He has had poison ivy. He has no undue susceptibility to infection. He has received penicillin for minor upper respiratory infections. After one such injection at age 27 he developed a swollen, painful, red ankle, thought to be due to a penicillin reaction.

Results

Members from three generations of a kindred were investigated by using the methods described above (Figure 1, Tables I and II). Individuals with decreased serum complement activity were found in each of the three generations.

Hemolytic complement titers of the sera of family members. The propositus (III, 7) and his sister (III, 10) and brother (III, 11) were found to have no demonstrable serum hemolytic activity. The sera of their spouses were found to have normal hemolytic complement activity (38 to 42 C'H₅₀ U per ml). The offspring of these matings (IV, 3 to 13) were found to have serum hemolytic complement titers that were below the lower limit of normal (20 to 24 C'H₅₀ U per ml Table II). A sister of the propositus whose serum contained 22 C'H₅₀ U married an individual with a normal serum complement titer. Of their two children, a boy (IV, 1) has a normal serum complement titer, and the serum of the other, a girl (IV, 2) has 24 C'H₅₀ U per ml.

The sera of the mother (II, 5) of the propositus and a maternal aunt (II, 6) contain 24 and 28 C'H₅₀ U per ml, respectively. A maternal aunt (II, 4) and two maternal uncles (II, 7 and 9) have normal serum hemolytic complement activity. The two daughters of one of them (III, 2 and 3) also have normal serum complement titers.

The father of the propositus (II, 3) and his two

¹⁰ Diagnostic tube antigen, *Br. abortus*; Sylvana Chemical Co., Orange, N. J.

¹¹ Agglutinating serum, *Br. abortus*; Burroughs Wellcome & Co., Tuckahoe, N. J.

C'2 DEFICIENCY

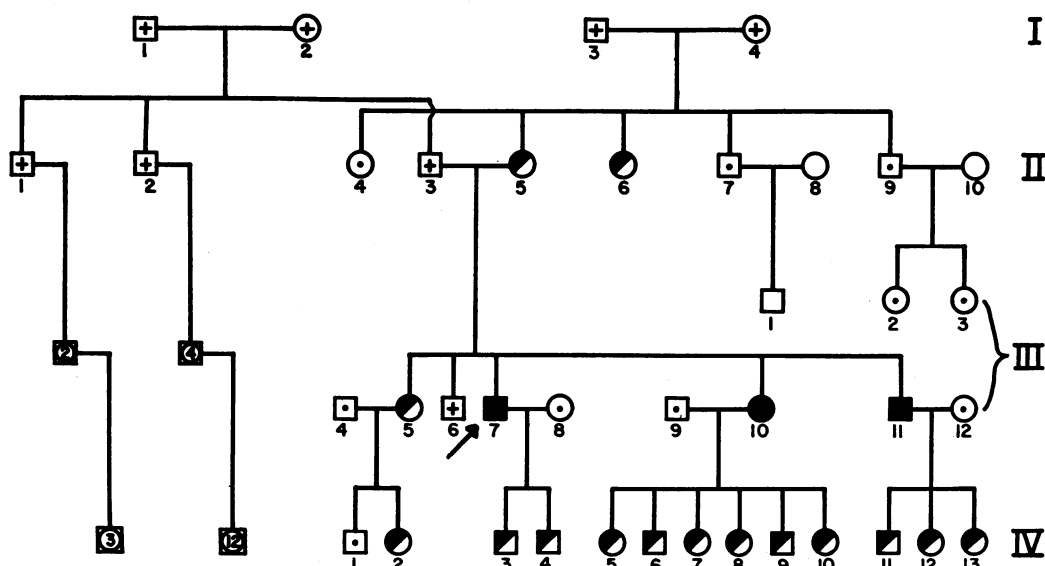


FIG. 1. PEDIGREE OF COMPLEMENT (C') DEFICIENCY IN MAN. (■—●) = deficient in second component of complement (C'2); (◐—◑) = partially deficient in C'2; (□—○) = normal level of C'2; (⊕—⊕) = deceased; and (□) = normal males and females. The number tested is given inside symbol. The arrow indicates the proband (III, 7).

brothers (II, 1 and 2) are deceased. The children of one of them (II, 2) have normal serum hemolytic complement activity. The maternal and paternal grandparents (I, 1 to 4) of the proband are deceased.

Titration of the serum complement components. When sera from members of this family were studied for C'2 activity, results consistent with those obtained by measuring the total serum hemolytic complement titers were noted (Tables I and II). As determined by the reagent method, three individuals (III, 7, 10, and 11) had C'2 titers of 3 to 6 U C'2 (normal = 128 U), and 15 (II, 5 and 6; III, 5; IV, 2 to 13) had subnormal titers ranging from 24 to 64 U C'2. Individuals who had normal CH_{50} were found to have normal C'2 titers by the reagent method (II, 4, 7, and 9; III, 2 and 3; IV, 1). When C'2 activity was measured with the EAC'la^{sp},4^{sp} cell assay, concordant results were obtained (Tables I and II). Three individuals each had C'2 activity of less than 3 U per ml (normal = 350 to 670). Fifteen individuals had subnormal levels (184 to 316 U per ml). Individuals with

normal serum hemolytic activity had normal C'2 activities with the EAC'la^{sp},4^{sp} assay.

The t_{max} , the time at which the greatest number of sites are in the SAC'la^{sp},4*,2^{hu} state, was determined by using C'2 deficient human serum as the source of C'2. The number of SAC'la^{sp},4*,2^{hu} sites per cell was determined from samples taken from 30 seconds' to 28 minutes' incubation. At no time was even one SAC'la^{sp},4*,2^{hu} site per cell formed (Figure 2). At 2 minutes of incubation time a minimal amount of SAC'la^{sp},4*,2^{hu} formation was measured in the C'2 deficient serum.

To ascertain whether any of the three other classical complement components were involved in the hemolytic defect, we performed titrations using the classical reagent method. In all sera tested, the titers of C'1, C'3, and C'4 were found to be normal (Table I).

The effect of adding highly purified C'2 to C'2 deficient serum. An amount of highly purified human C'2 calculated to restore the serum complement activity to a normal level was added to C'2 deficient serum. The complement activity of the

TABLE I
Serum complement studies on the *propositus* and his siblings and mother*

Individual†	C'H ₅₀	EAC'la ^{sp} ,4 ^{sp} C'2	Reagent C'2	Reagent C'1	Reagent C'3	Reagent C'4	Serum β ₂ -globulin	Serum bactericidal titer	C'I-A ₃₀	EI
	U/ml	U/ml	U/ml	U/ml	U/ml	U/ml	mg/100 ml		U/ml	mg/100 ml
II, 5	24	256	24	1,836	192	1,024	ND	1:15	568	2.7
III, 5	22	275	48	1,836	128	1,024	164	1:15	568	2.7
III, 7	0	<3	6	1,836	192	1,024	150	1:3	130	2.6
III, 10	0	<3	4	1,836	192	1,836	147	1:3	32	2.1
III, 11	0	<3	3	1,836	192	1,024	ND	1:3	48	ND
Normal	32-45	350-670	96-192	1,024-2,048	128-192	1,024-1,836	130-155	1:20-1:30	760-1,440	2.4 ± 0.4

* Abbreviations: C'H₅₀ = serum hemolytic complement titer; EAC'la^{sp},4^{sp} = guinea pig first and fourth complement components; C'I-A₃₀ = immune adherence titer; EI = esterase inhibitor; and ND = not determined.

† All determinations on C'2 deficient sera were performed two or more times depending on the availability of the sera. All normal values are based on at least 50 assays.

reconstituted serum was then measured by hemolytic assay, and the serum was found to contain 36 C'H₅₀ U per ml (Table III). When the reconstituted serum was assayed for C'2 activity utilizing the EAC'la^{sp},4^{sp} cell titration, a normal value (339 U) was also obtained (Table III). When functionally pure guinea pig C'2 was added to a 1:40 dilution of C'2 deficient human serum 1 C'H₅₀ U was restored with 16 U of guinea pig C'2.

In order to ascertain whether or not C'2 deficient serum blocked the interaction of hemolytically active C'2 with SAC'la,4 sites, we added a 1:10 dilution of serum from an individual deficient in C'2 to functionally pure guinea pig C'2 and measured the resulting C'2 activity by the stoichiometric EAC'la^{sp},4^{sp} cell titration. The resulting C'2 titer was identical to that obtained when functionally pure guinea pig C'2 was diluted with the same amount of saline. This experiment was repeated with normal human serum as the source of C'2. The same results were obtained; there was no change in C'2 activity when C'2 deficient human serum was added (Figure 3). Thus, the C'2 deficient serum did not appear to contain an inhibitor or an altered protein that could attach to SAC'la^{sp},4^{sp} sites and block the activity of guinea pig or human C'2.

TABLE II
Serum hemolytic complement and C'2 titers of individual members of the kindred

Individual	C'H ₅₀	EAC'la ^{sp} ,4 ^{sp} C'2	Reagent C'2
	U/ml	U/ml	U/ml
II, 4	39	551	128
II, 6	28	316	48
II, 7	39	562	128
II, 9	43	488	128
III, 2	40	520	128
III, 3	38	617	128
III, 4	33	556	128
III, 8	42	440	128
III, 9	39	514	128
III, 12	38	402	128
IV, 1	36	538	128
IV, 2	24	234	48
IV, 3	22	198	48
IV, 4	22	231	64
IV, 5	21	184	48
IV, 6	24	202	32
IV, 7	ND	253	ND
IV, 8	21	180	48
IV, 9	ND	188	48
IV, 10	ND	277	ND
IV, 11	20	172	32
IV, 12	24	249	32
IV, 13	21	225	32

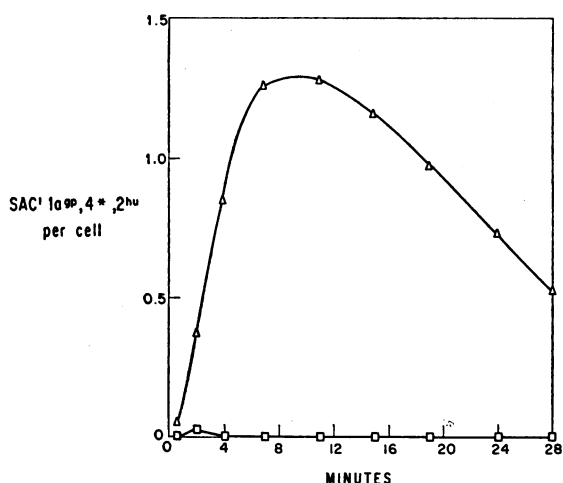


FIG. 2. KINETICS OF SAC'1A^{sp}, 4^{*}, 2^{hu} FORMATION. The normal serum (Δ) was diluted 1:500; the serum from a C'2 deficient individual (□) was diluted 1:10. SAC'-1a^{sp}, 4^{*}, 2^{hu} = sites formed by the interaction of whole human serum with guinea pig first and fourth complement components.

Immunodiffusion studies of the serum beta_{1C}-globulin (C'3), beta_{1E}-globulin (C'4), beta_{1F}-globulin (C'5), and C'1 esterase inhibitor. The sera of both C'2 deficient individuals (III, 7 and 10) and an individual with a subnormal C'2 titer (III, 5) contained normal amounts (130 to 155 mg per 100 ml) of beta_{1C}-globulin when measured by a quantitative immunodiffusion technique (Table I). When the serum of a C'2 deficient individual (III, 10) was investigated by immunoelectrophoresis against antisera identifying human serum beta_{1C}-globulin, beta_{1E}-globulin, and beta_{1F}-globulin, we found that normal bands of precipitation were developed for these three globulins.

When a washed immune gamma globulin-anti-gamma globulin precipitate was added to C'2 deficient human serum, conversion of beta_{1C}-globulin occurred. The conversion was about 75%

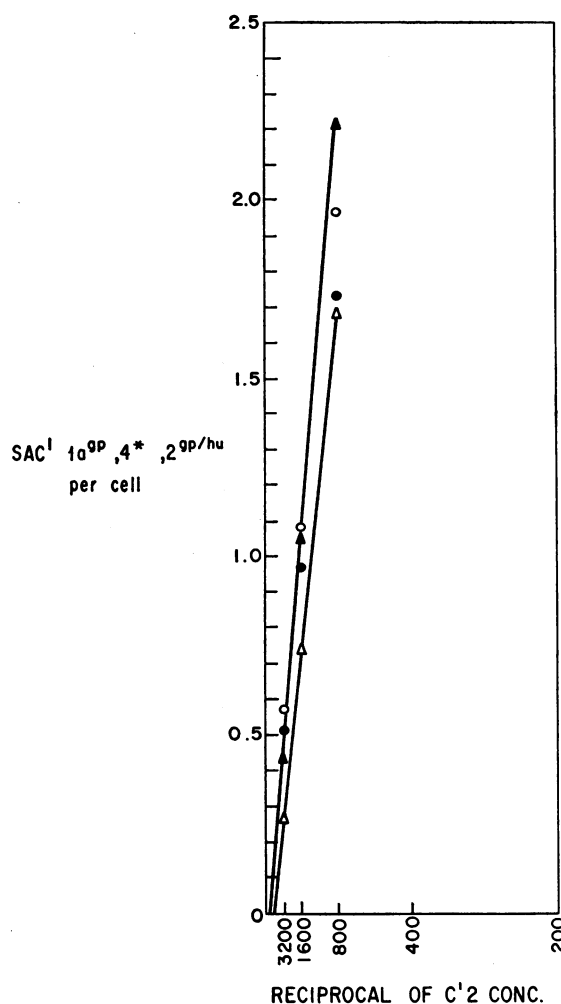


FIG. 3. EFFECT OF C'2 DEFICIENT HUMAN SERUM ON THE TITRATION OF FUNCTIONALLY PURE GUINEA PIG C'2 AND C'2 IN NORMAL HUMAN SERUM. (○) = titration of functionally pure guinea pig C'2; (●) = titration of functionally pure guinea pig serum mixed with C'2 deficient human serum; (Δ) = titration of normal human serum; and (▲) = titration of normal human serum mixed with C'2 deficient serum.

TABLE III
The effect of adding highly purified human C'2 to C'2 deficient serum

	C'2 deficient serum	C'2 deficient serum with added human C'2
C'H ₅₀ , U/ml*	0	36
C'2, U/ml*	<3	339
C'I-A ₅₀ , U/ml*	66	1,390
Bactericidal titer*	1:3	1:25

* See Table I for normal serum values.

complete after 2 hours of incubation at 37° C. The beta_{1C}-globulin in a normal control serum was completely converted within 20 minutes. The addition of EDTA at 0.01 M final concentration completely inhibited the conversion of beta_{1C}-globulin in both the C'2 deficient and the normal sera.

To ascertain that the deficiency in the kindred described was not an asymptomatic variant of hereditary angioneurotic edema, both quantitative immunodiffusion and functional determinations of C'1 esterase inhibitor were performed. The level

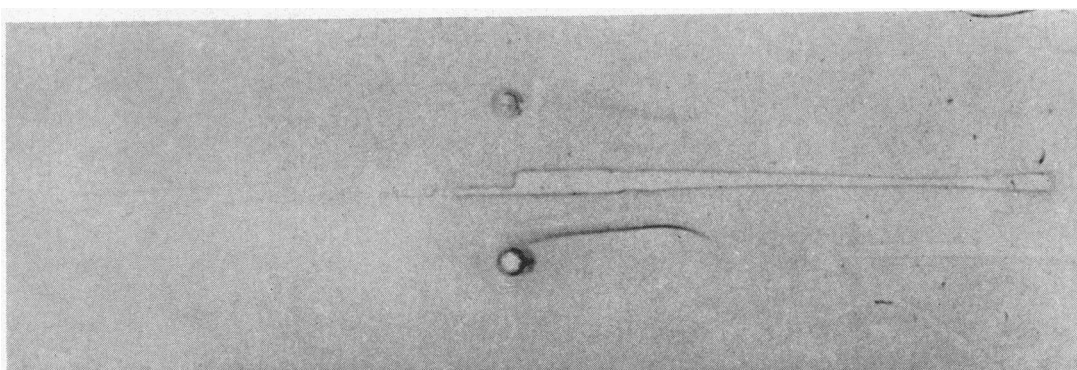


FIG. 4. IMMUNOELECTROPHORETIC PATTERN OF C'1 ESTERASE INHIBITOR IN SERUM. The top well was filled with serum from a patient with hereditary angioneurotic edema (HANE). The lower, with serum from a C'2 deficient individual. The trough was filled with rabbit anti-C'1 esterase inhibitor. The HANE serum formed no precipitation band, whereas a precipitation band was formed with the C'2 deficient serum.

of this protein was found to be normal (2.4 ± 0.4 mg per 100 ml) in both C'2 deficient and C'2 intermediate individuals (Table I). The serum from a C'2 deficient individual yielded a normal pattern in immunoelectrophoresis against rabbit antihuman C'1 esterase inhibitor (Figure 4). The functional serum C'1 esterase inhibitor titer of this serum was normal, 4.5 U per ml.

Serum bactericidal activity of family members. The sera of three C'2 deficient individuals (III, 7, 10, and 11) had demonstrable but decreased *in vitro* bactericidal activity against *S. typhosa* 0901. A 50% bactericidal end point was obtained with a serum dilution 1:3 (normal = 1:25, Table I). Two sera with subnormal C'2 (II, 5 and III, 5) had bactericidal titers of 1:15 (Table I). C'2 deficient serum reconstituted with highly purified

human C'2 to a normal hemolytic activity and C'2 titer also had normal bactericidal activity, 1:25.

Serum complement activity of family members as determined by the method of immune-adherence (C'I-A₃₀). The sera of three C'2 deficient individuals (III, 7, 10, and 11) were found to have activity of one-ninth to one-fortieth of normal (760 to 1,440 U per ml, Figure 5). Two individuals with subnormal serum C'2 (II, 5 and III, 5) had more than one-half of normal immune-adherence activity, a finding similar to that obtained with the hemolytic complement titration (Figure 5, Table I). C'2 deficient serum reconstituted with highly purified human C'2 so that it then contained normal hemolytic activity and C'2 titer also had normal immune-adherence activity (1,390 U per ml).

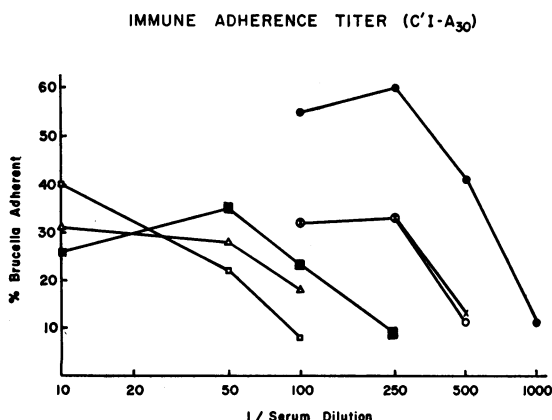


FIG. 5. TITRATION OF SERUM COMPLEMENT BY IMMUNE ADHERENCE (C'I - A₃₀). (□, △, ■) are C'2 deficient; (○, ×) are partially C'2 deficient; (●) is normal.

Discussion

A hereditary deficiency of the second component of complement in a kindred is described in this report; the defect has been identified in members from three generations. The sera of three siblings are almost totally deficient in C'2 activity. The sera of the mother, a maternal aunt, a sister, a niece, and the 11 children of the individuals deficient in C'2 were found to be partially deficient in C'2. The defect appears to be transmitted as an autosomal recessive characteristic, although individuals who are heterozygous for the defect are easily detectable by virtue of the fact that their sera have approximately half the normal serum complement activity and C'2 titers. Because no gene product of the abnormal allele is thus far dis-

cernible, codominance of the alleles is not established. It is not technically feasible at present to determine whether or not any cross-reacting material (40) is synthesized as a gene product or the abnormal allele. However, we have shown in this report that C'2 deficient sera do not contain a gene product that functionally competes or interferes with normal hemolytically functional C'2.

Sera deficient in hemolytic C'2 were also found to be defective in two other complement dependent systems, immune adherence and bacteriolysis. Sera from individuals homozygous for the C'2 deficiency had 10 to 15% of normal immune adherence and bactericidal activity, whereas sera of heterozygous individuals contained 50% of the normal activity in these two complement dependent systems. Furthermore, the conversion of β_{1C} -globulin to β_{1G} -globulin, which has been considered to be a C'2 dependent reaction, has been demonstrated in C'2 deficient serum, although the conversion proceeds at a very retarded rate. It is possible that the C'2 deficient sera contain sufficient C'2 molecules to effect detectable activity in the possibly more sensitive immune adherence and bactericidal assays, whereas they would be functionally undetected in the hemolytic assay used here. Alternatively, other pathways may exist to the activation of classical third component activity with resultant detectable bactericidal and immune-adherence activity. The hemolytic, bactericidal, and immune adherence activities of C'2 deficient serum were fully restored upon the addition of highly purified human C'2.⁵ These observations suggest that the sera are C'2 deficient by virtue of a single error of protein biosynthesis, and that the protein is functionally operative in the three complement dependent systems studied.

The existence of primary complement deficient states in man has been previously reported. Scarozzi (41) discussed seven individuals from three separate families who had markedly depressed total serum hemolytic complement activity. No additional investigations were performed to establish a specific component deficiency.

In 1960 Silverstein (42) reported findings from an individual with a decreased serum hemolytic complement titer. Reagent titrations of each of the four classical complement components established that the defect was associated with a specific decrease in C'2 activity. Studies on the se-

rum complement system of the individual's two children and both of his siblings yielded normal results, so that the evidence for genetic transmission was not established.

A study involving the measurement of hemolytic serum complement activity of 41,082 Swiss army recruits was carried out by Hässig and his colleagues (43). They noted that 14 individuals were deficient in serum hemolytic complement activity. With a complement reagent method, a decrease in C'2 or C'4 was found in the sera of the majority of these individuals. The medical histories of these individuals did not establish any underlying illness or yield any information to distinguish these recruits from others. No specific attempt to exclude hereditary angioneurotic edema as the basis for the decreased serum hemolytic complement titer and for the decrease in C'2 and C'4 was reported by the authors. The families of 13 of the recruits were investigated, but no definite hereditary pattern could be established in any kindred.

Kumate (44) has recently studied two brothers, one of whom had recurrent bacterial meningitis. Both boys had a decrease in serum hemolytic complement activity, and the defect was found to be limited to C'2. Six other siblings and both parents of the *propositus* were found to have normal serum hemolytic complement activity and normal C'2 titers. Whereas no definite genetic transmission could be established in this family, the possibility of an autosomal recessive mode of transmission with only those homozygous for the abnormal gene having a detectable defect is not excluded.

In man the only genetically determined abnormality of the serum complement system thus far established is hereditary angioneurotic edema. Donaldson and co-workers (18, 35) have shown that there is a hereditary biosynthetic defect in the inhibitor of the esterase of C'1 in this disease. During attacks of hereditary angioneurotic edema activated C'1 esterase is demonstrable in the plasma of the patients, and a concomitant diminution of C'2 and C'4 titers is demonstrable (18, 27). The sera of the kindred reported here have normal C'1 esterase inhibitor and C'4 titers: consequently, the C'2 deficient individuals do not have hereditary angioneurotic edema.

Several heritable complement deficiencies are known in laboratory animals. Moore (21) de-

scribed a complement deficient strain of guinea pigs. The defect was transmitted as an autosomal recessive trait and was found to involve the classical C3 complex as defined by assays employing reagent methods (45). In 1961, Rother and Rother (19) found a strain of complement deficient rabbits. Rother and Rother found the sera of these rabbits to lack C'6 (46). As yet the exact genetic transmission of the defect has not been established.

Two strains of inbred mice deficient in hemolytic serum complement were reported by Rosenberg and Tachibana in 1962 (20). The defect has been demonstrated to be limited to C'5 (47). Since the initial report several other inbred strains of mice have been found that lack serum hemolytic complement activity (48). The hemolytic defect appears to be transmitted genetically as an autosomal recessive trait determined by the gene locus Hc⁰. Mice possessing the allelic gene locus Hc¹ have hemolytic complement activity, and no gene-dose phenomenon has been noted (49).

The relative freedom from bacterial infection of the three C'2 deficient individuals is consistent with experiments performed with C'5 deficient mice and C'6 deficient rabbits. Stiffel and his co-workers (50) have reported that complement deficient mice have normal *in vivo* bactericidal activity when challenged with intravenously administered *Salmonella typhimurium*. Similar results were obtained by Rother and Rother with complement deficient rabbits challenged intravenously with *S. typhosa*. When the sera from complement deficient rabbits were used in an *in vitro* bactericidal assay against *S. typhosa*, no bactericidal activity could be demonstrated (45). In both of the *in vivo* experiments the injected bacteria were phagocytosed principally by the reticuloendothelial cells of the liver.

The three homozygous C'2 deficient individuals in this kindred are in apparent good health and have manifested both delayed hypersensitivity (poison ivy, tuberculin skin test) and penicillin-induced serum sickness. The role of complement in the defense mechanisms of the intact organism remains unclarified.

Summary

A hereditary deficiency of the second component of complement in man has been established. The

abnormality is defined by a virtual absence of hemolytic activity measured either as whole complement (C'H₅₀) or C'2. Titrations of C'1, C'3, and C'4 by the classical reagent methods are normal in affected individuals. Levels of serum C'1 esterase inhibitor, beta_{1C}-globulin (C'3), beta_{1F}-globulin (C'5), and beta_{1E}-globulin (C'4), are also normal. Immune-adherence complement titers using a *Br. abortus* system are severely decreased as is the *in vitro* serum bactericidal activity against *S. typhosa*. Evidence is presented which indicates that the defect is caused by a biosynthetic defect of the functional protein.

Acknowledgments

We are grateful to Dr. Virginia H. Donaldson for performing the functional C'1 esterase inhibitor determination; to Dr. J. Gabriel Michael for his guidance in performing the serum bactericidal assays; and to Dr. Hurston F. Heslington, Florence, Ala., Dr. Louis Gillespie, Washington, D.C., Dr. J. Lawrence Naiman, Philadelphia, Pa., and Dr. Robert C. Skarnes, Boston, Mass., for helping to obtain serum specimens. We are greatly indebted to Dr. Irwin H. Lepow, Dept. of Medicine, Western Reserve University, Cleveland, Ohio, for highly purified human C'2 and to Dr. Manfred M. Mayer, Dept. of Microbiology, The Johns Hopkins University, Baltimore, Md., for functionally pure guinea pig C'2.

References

1. Mayer, M. M. Mechanism of hemolysis by complement *in* Complement, G. E. W. Wolstenholme and J. Knight, Eds. London, J. & A. Churchill, 1965, pp. 4-32.
2. Nelson, R. A., Jr. The role of complement in immune phenomena *in* The Inflammatory Process, B. W. Zweifach, L. H. Grant, and R. T. McCluskey, Eds. New York, Academic Press, 1965, pp. 819-872.
3. Mayer, M. M. On the destruction of erythrocytes and other cells by antibody and complement. *Cancer Res.* 1961, 21, 1262.
4. Muschel, L. M., and H. P. Treffers. Quantitative studies on the bactericidal actions of serum and complement. II. Some implications for the mechanism of the bactericidal reaction. *J. Immunol.* 1956, 76, 11.
5. Rother, K., U. Rother, K. F. Petersen, D. Gerns, and F. Mitze. Immune bactericidal activity of complement. Separation and description of intermediate steps. *J. Immunol.* 1964, 93, 319.
6. Green, H., R. A. Fleischer, P. Barrow, and B. Goldberg. The cytotoxic action of immune gamma globulin and complement on Krebs ascites tumor cells. II. Chemical studies. *J. exp. Med.* 1959, 109, 511.

7. Ross, A., and I. H. Lepow. Studies on immune cellular injury. I. Cytotoxic effects of antibody and complement. *J. exp. Med.* 1960, **112**, 1085.
8. Nelson, R. A. Immune-adherence in Mechanism of Cell and Tissue Damage Produced by Immune Reactions, P. Grabar and P. Miescher, Eds. Basel, Benno-Schwabe, 1962, pp. 245-248.
9. Nelson, D. S. Immune adherence in Complement, G. E. W. Wolstenholme and J. Knight, Eds. London, J. & A. Churchill, 1965, pp. 222-237.
10. Ecker, E. E., and G. Lopez-Castro. Complement and opsonic activities of fresh human sera. *J. Immunol.* 1947, **55**, 169.
11. Ward, P. A., and C. G. Cochrane. The role of serum complement in chemotaxis *in vitro*. *Fed. Proc.* 1965, **24**, 447.
12. Ward, P. A., C. G. Cochrane, and H. J. Müller-Eberhard. Role of serum complement in chemotaxis of leukocytes *in vitro*. *J. exp. Med.* 1965, **122**, 327.
13. Lange, K., F. Graig, J. Oberman, L. Slobody, G. Ogur, and F. LoCasto. Changes in serum complement during the course and treatment of glomerulonephritis. *Arch. intern. Med.* 1951, **88**, 433.
14. Wedgwood, R. J. P., and C. A. Janeway. Serum complement in children with "collagen diseases." *Pediatrics* 1953, **11**, 569.
15. Fischel, E. E., and D. C. Gajdusek. Serum complement in acute glomerulonephritis and other renal diseases. *Amer. J. Med.* 1952, **12**, 190.
16. Lange, K., E. Wasserman, and L. B. Slobody. The significance of serum complement levels for the diagnosis and prognosis of acute and subacute glomerulonephritis and lupus erythematosus disseminatus. *Ann. intern. Med.* 1960, **53**, 636.
17. Miescher, P. A., F. Paronetto, and D. Koffler. Immune studies in vasculitis associated with 19 S-7 S type of cryoglobulinemia (abstract). *J. clin. Invest.* 1965, **44**, 1075.
18. Donaldson, V. H., and F. S. Rosen. Action of complement in hereditary angioneurotic edema: the role of C'1-esterase. *J. clin. Invest.* 1964, **43**, 2204.
19. Rother, K., and U. Rother. Über einen angeborenen Komplement-Defekt bei Kaninchen. *Z. Immun.-Forsch.* 1961, **121**, 225.
20. Rosenberg, L. T., and D. K. Tachibana. Activity of mouse complement. *J. Immunol.* 1962, **89**, 861.
21. Moore, H. D. Complementary and opsonic functions in their relation to immunity. A study of the serum of guinea-pigs naturally deficient in complement. *J. Immunol.* 1919, **4**, 425.
22. Woodworth, H. C. Unpublished observations.
23. Kabat, E. A., and M. M. Mayer. Complement and complement fixation in Experimental Immunochimistry, 2nd ed. Springfield, Ill., Charles C Thomas, 1961, pp. 133-240.
24. Public Health Monograph No. 74. Standardized Diagnostic Complement Fixation Method and Adaptation to Micro Test. Washington, D. C., U. S. Government Printing Office, 1965, p. 12.
25. Wedgwood, R. J. Measurement of the components of complement by the reagent titration technique. *Z. Immun.-Forsch.* 1959, **118**, 358.
26. Austen, K. F., and F. Beer. The measurement of second component of human complement (C'2^{hu}) by its interaction with EAC'1a^{sp}, 4^{sp} cells. *J. Immunol.* 1964, **92**, 946.
27. Austen, K. F., and P. S. Russell. Detection of renal allograft rejection in man by demonstration of a reduction in the second component of human complement. *Ann. N. Y. Acad. Sci.* In press.
28. Austen, K. F., and A. L. Sheffer. Detection of hereditary angioneurotic edema by demonstration of a reduction in the second component of human complement. *New Engl. J. Med.* 1965, **272**, 649.
29. Lepow, I. H., E. W. Todd, R. D. Smink, Jr., and J. Pensky. Purification of the second component (C'2) of human complement. *Fed. Proc.* 1965, **24**, 446.
30. Borsos, T., H. J. Rapp, and C. T. Cook. Studies on the second component of complement. III. Separation of the second component from guinea pig serum by chromatography on cellulose derivatives. *J. Immunol.* 1961, **87**, 330.
31. Scheidegger, J. J. Une micro-méthode de l'immuno-électrophorèse. *Int. Arch. Allergy* 1955, **7**, 103.
32. Müller-Eberhard, H. J., U. Nilsson, and T. Aronsson. Isolation and characterization of two β_1 -glycoproteins of human serum. *J. exp. Med.* 1960, **111**, 201.
33. Pensky, J. Further purification of a human serum inhibitor C'1-esterase. *Fed. Proc.* 1960, **19**, 76.
34. Klempner, M. R., S. P. Gotoff, C. A. Alper, A. S. Levin, and F. S. Rosen. Estimation of the serum beta_{1c} globulin concentration: its relation to the serum hemolytic complement titer. *Pediatrics* 1965, **35**, 765.
35. Rosen, F. S., P. Charache, J. Pensky, and V. Donaldson. Hereditary angioneurotic edema. Two genetic variations. *Science* 1965, **148**, 957.
36. Levy, L. R., and I. H. Lepow. Assay and properties of serum inhibitor of C'1-esterase. *Proc. Soc. exp. Biol. (N. Y.)* 1959, **101**, 608.
37. Michael, J. G., J. L. Whitby, and M. Landy. Studies on natural antibodies to gram-negative bacteria. *J. exp. Med.* 1962, **115**, 131.
38. Woodworth, H. C. Studies with immune-adherence. I. Titration of human complement with a rice starch system. *Immunology* 1962, **5**, 181.
39. Nishioka, K. Measurements of complement by agglutination of human erythrocytes reacting in immune-adherence. *J. Immunol.* 1963, **90**, 86.
40. Suskind, S. R., C. Yanofsky, and D. M. Bonner. Allelic strains of neurospora lacking tryptophan synthetase: a preliminary immunochemical char-

- acterization. Proc. nat. Acad. Sci. (Wash.) 1955, 41, 577.
41. Scartozzi, C. Pouvoir complementaire faible à caractère familial. Soc. internaz. di microbiol., Boll. d. sez. ital. 1937, 9, 351.
 42. Silverstein, A. M. Essential hypocomplementemia. Report of a case. Blood 1960, 16, 1338.
 43. Hässig, A., J. F. Borel, P. Ammann, M. Thöni, and R. Büttler. Essentielle hypokomplementämie. Path. et Microbiol. (Basel) 1964, 27, 542.
 44. Kumate, J. Meningitis repetidas asociadas con hipocomplementemia. Presented at the Fifteenth Meeting of the Asociación de Investigación Pediátrica, Mexico, D. F., 1962.
 45. Hyde, R. R. The complement deficient guinea pig: a study of an inheritable factor in immunity. Amer. J. Hyg. 1932, 15, 824.
 46. Rother, K., and U. Rother. Studies on complement defective rabbits. IV. Blood clearance of intravenously injected *S. typhi* by the reticuloendothelial system. Proc. Soc. exp. Biol. (N. Y.) 1965, 119, 1055.
 47. Nilsson, U., and H. J. Müller-Eberhard. Immunologic relation between human β_{1F} -globulin and mouse MuBI(HC). Fed. Proc. 1965, 24, 620.
 48. Cinader, B., S. Dubiski, and A. C. Wardlaw. Distribution, inheritance and properties of an antigen, MuBI, and its relation to hemolytic complement. J. exp. Med. 1964, 120, 897.
 49. Herzenberg, L. A., D. K. Tachibana, L. A. Herzenberg, and L. T. Rosenberg. A gene locus concerned with hemolytic complement in *Mus musculus*. Genetics 1963, 48, 711.
 50. Stiffel, C., G. Biozzi, D. Moulton, Y. Bouthillier, and C. Decreusefond. Studies on phagocytosis of bacteria by the reticulo-endothelial system in a strain of mice lacking hemolytic complement. J. Immunol. 1964, 93, 246.