Hereditary Deficiency of the Sixth Component of Complement in Man

I. IMMUNOCHEMICAL, BIOLOGIC, AND FAMILY STUDIES

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ABSTRACT An 18-yr-old black woman in good general health was found to lack serum hemolytic complement activity. The sixth component of complement (C6) was undetectable by functional assay of serum or plasma and by immunoprecipitin analysis of serum. Functional titers of all other complement components were normal. The absence of C6 in the patient's serum could not be accounted for by a circulating C6 inhibitor, and addition of functionally pure C6 to the patient's serum restored hemolytic activity to normal. Both parents of the proband and five of six available siblings had approximately half the normal levels of functional C6. The other sibling had a normal C6 level. These data suggest that both parents and five siblings are heterozygous for C6 deficiency, while the proband is homozygous and one sibling is normal. Thus, C6 deficiency appears to follow classic mendelian inheritance, with all three possible genotypes recognizable within the family.

Functional properties of the proband's C6-deficient serum included total absence of bactericidal activity against *Salmonella typhi 0 901* and *Hemophilus influenzae, type b,* and inability to mediate lysis of red blood cells from patients with paroxysmal nocturnal hemoglobinuria in either the acidified serum or "sugar water" tests. The proband's serum did, however, exhibit a normal capacity (*a*) to generate chemotactic activity during incubation with bacterial endotoxin or aggregated IgG, (*b*) to mediate the immune adherence phenomenon, and (*c*) to coat human red blood cells, sensitized by cold agglutinins, with C4 and C3.

This study was presented in part at the meeting of the American Society for Clinical Investigation, Atlantic City, N. J., 29 April 1973 (1973. *J. Clin. Invest.* **52**: 50*a*. [Abstr.]) and at the 5th International Complement Workshop, Coronado, Calif., 25 February 1973.

Received for publication 28 June 1973 and in revised form 19 September 1973.

INTRODUCTION

Recent studies of complement deficiency states in man and experimental animals have contributed greatly to our understanding of the role of the complement (C)[1] system in host defense, inflammation, and tissue injury (1, 2). Human families have been identified with genetically controlled deficiency of the second C component (C2) (3–6) or of the C1r subcomponent of C1 (7, 8), two proteins which act early in the "classical" sequence of C activation (1). An apparently high proportion of individuals with these hereditary C deficiencies have been reported to exhibit rheumatic disorders, renal disease, and autoimmune phenomena (7–11). Patients with C3 deficiency and abnormalities of C5 function have also been described, and both of these disorders have been associated with increased susceptibility to pyogenic infection (12–15).

[1] *Abbreviations used in this paper:* A, antisheep erythrocyte antibody; C, complement; $C1_{GP}$, guinea pig C1; $C4_{HU}$, human C4; DVBS, dextrose-Veronal-buffered saline; EA, antibody-sensitized erythrocytes; LE, lupus erythematosus; PBS, phosphate-buffered saline; PNH, paroxysmal nocturnal hemoglobinuria; RBC, red blood cell; VDRL, Venereal Disease Research Laboratory.

It is the purpose of this report to describe a new hereditary deficiency of one of the late-acting components in the C sequence, C6, in a human kindred and to examine the functional properties of C6-deficient human serum. This defect is as yet unassociated with definite clinical illness.

CASE SUMMARY

D. B., an 18-yr-old married black female, was admitted to the University of Rochester Medical Center in August 1971 with a 1-day history of fever, chills, polyarthralgia, and painful fingertip lesions. The patient denied vaginal discharge but did describe occasional left lower abdominal pain in the preceding week. Past health had been excellent except for admission to another hospital 13 mo earlier for gonococcal arthritis which had responded to penicillin therapy.

Examination revealed rectal temperature 101.4°F and extremely tender, raised, erythematous 5-mm skin lesions on the tips of both index fingers, consistent with hemorrhagic infarcts. There was tenderness and slight swelling of several small joints of both hands and the right foot, as well as tenosynovitis affecting several tendon groups in the left leg. The abdomen was negative. Pelvic examination revealed white, mucoid vaginal discharge, tenderness of the uterus and adnexa, but no masses.

A positive culture for *Neisseria gonorrheae* was obtained from the uterine cervix. Three blood cultures were negative and a rectal culture showed normal flora. Other laboratory data included: hematocrit 34%; white blood cells 7,400 with a slight "left shift"; normal platelets on smear; negative urine analysis; negative Venereal Disease Research Laboratory (VDRL) test for syphilis; normal blood chemistries including bilirubin, alkaline phosphatase, lactic dehydrogenase, glutamic-oxaloacetic transaminase, urea nitrogen, creatinine, uric acid, total proteins, and albumin. A screening test revealed deficiency of red blood cell (RBC) glucose-6-phosphate dehydrogenase. Because the original differential diagnosis included lupus erythematosus (LE), tests for antinuclear antibody, LE cells, and serum complement were obtained. The former two tests were negative but the hemolytic complement (CH_{50}) titer was < 5 U/ml (normal range 80–160). This was confirmed on several additional samples of fresh serum, including one obtained after the patient had fully recovered.

A diagnosis of gonorrhea with probable gonococcemia was made. Treatment with large doses of penicillin plus probenecid was followed by rapid clearing of fever and other symptoms, and the patient has remained well during subsequent observation as an outpatient.

When it became clear that the extremely low CH_{50} titer represented a specific deficiency of C6 (see below), the patient was recalled for a more directed clinical history and examination and for clotting studies (16). Common childhood infections as well as smallpox vaccination had been handled uneventfully, and there had been no problem with infectious diseases other than gonorrhea. The patient denied petechiae, easy bruising, gingival bleeding, or excessive menstrual flow. 2½ yr earlier three teeth had been extracted without excessive bleeding. Other than the gonococcal disease, there was no history or findings suggestive of joint disease, muscle pain or weakness, pleurisy, kidney disease, proteinuria, or skin lesions. The patient did relate, however, that during the past 2 yr her hands and feet became cold and somewhat numb in cold weather, or when she handled cold objects. She denied blanching, discoloration, pain, or ulcerations in the affected areas.

The family history also was negative for infectious, rheumatic, or bleeding disorders. Two younger siblings had died in infancy, one attributed to brain damage at birth and the other to a "heart condition." The other seven siblings are well. The patient's mother had exhibited hypertension during pregnancy and was treated for bronchopneumonia in 1967. The father's health had been excellent. The parents denied consanguinity.

During this period additional laboratory studies on the proband showed: hemoglobin 13.1 g/100 ml; platelet count 198,000/mm^3; repeat VDRL test negative; repeat test for antinuclear antibody negative; and serum latex fixation test for rheumatoid factor negative. A test for cryoglobulins was weakly positive on two occasions (1.1% and 0.5% cryocrit). The washed, redissolved cryoglobulin (see Methods) gave a positive slide flocculation reaction with IgG-latex particles and, by immunodiffusion, contained mainly IgG plus small amounts of IgM and IgA, and no albumin. Using whole serum separated at 37°C, IgG, IgA, and IgM concentrations were 1,165, 96, and 128 mg/100 ml, respectively, by radial immunodiffusion (normal ranges: IgG 700–2,000, IgA 50–300, IgM 50–250). After removal of the cryoglobulins the supernatant serum, run concurrently, gave the following values: IgG 1,070, IgA 94, and IgM 117 mg/100 ml, respectively.

METHODS

Human sera. Human sera for C assays were either freshly drawn or stored as small aliquots at −70°C and discarded after one thaw. Such frozen samples from D. B. and her family members, together with normal control sera, were shipped or personally delivered to NIH in Dry Ice packs.

Specific antisera. Specific antisera to human IgM, IgG, and IgA were prepared in rabbits as previously described (17). Rabbit antisera to human C3 and C4 were the gift of Dr. John T. Boyer, University of Arizona School of Medicine, Tucson, Ariz. Antiserum to rabbit C6 was prepared by immunizing C6-deficient rabbits with normal rabbit serum in Freund's complete adjuvant (Difco Laboratories, Detroit, Mich.). Antirabbit C6 has been observed to cross-

react with human C6 (18). Monospecific goat antihuman C6 was a gift of Dr. H. J. Müller-Eberhard, Scripps Clinic and Research Foundation, La Jolla, Calif. Rabbit antiserum to purified human C2 was prepared as previously described (19). Antiserum to human C3 proactivator (glycine-rich β-globulin, factor B) was supplied by Dr. Chester A. Alper, Children's Hospital Medical Center, Boston, Mass. Goat antialbumin was purchased from Kallestad Laboratories, Inc., Minneapolis, Minn.

Complement reagents and assays. Plates for measuring human C3 and C4 by radial immunodiffusion were purchased from Hyland Div. Travenol Laboratories, Costa Mesa, Calif. and Behring Diagnostics, Inc., Somerville, N. J., respectively. Normal ranges were established with sera from 20 healthy donors. C6-deficient rabbit serum was obtained from rabbits purchased from Rancho de Conejo, La Jolla, Calif. Human C2-deficient serum (J. S.) was obtained from the proband of a new family with hereditary C2 deficiency, to be reported elsewhere. Sheep RBC were obtained from sheep of genetic type LK (LL).

Hemolytic C (CH_{50}) titers were determined as previously reported (20). Individual, functionally pure guinea pig and human complement components (C3-9) were obtained from the Cordis Corp., Miami, Fla. Sources of materials and buffers and methods for the preparation of cellular intermediates in the complement system have been described in detail (21, 22). Functional assays of each of the nine complement components were performed as previously described (23).

Assays of C6 were performed by two methods. In the first method, involving stepwise buildup of components on antibody sensitized erythrocytes (EA), human components were used throughout. To 0.25 ml of the test dilution in dextrose-Veronal-buffered saline (DVBS) (22) was added 0.25 ml of a reagent containing C3 (50 U/ml), C5 (50 U/ml), and C7 (50 U/ml). To this mixture were added 0.25 ml C2 (100 U/ml) and 0.25 ml EAC14 (1×10^8 cells/ml, $T_{max} = 2$ min). After incubating the reaction mixture at 30°C for 30 min, 0.25 ml of a mixture containing C8 (500 U/ml) and C9 (500 U/ml) were added and the reaction incubated for a further 60 min at 37°C. Then 2.5 ml ice-cold EDTA-buffer (isotonic Veronal buffer containing 0.01 M EDTA) were added and the degree of hemolysis determined. Controls included the various reagents in the absence of a serum C6 source, a functionally pure C6 source, and water-lysed cells to determine the absorbance at 100% hemolysis. Data were plotted according to the Poisson distribution and the one-hit theory of complement action (24). These C6 titrations were performed on sera from all members of the family and 14 normal controls with the same set of reagents to minimize error.

A second method was developed for C6 titration utilizing serum from rabbits genetically deficient in C6. A volume of 0.25 ml of sensitized sheep RBC, containing 1.5×10^8 cells/ml in DVBS, was mixed with 0.25 ml C6-deficient rabbit serum diluted 1/20 in DVBS and the mixture incubated at 37°C for 30 min. To the mixture was added 0.25 ml of a dilution of the C6 source in the same buffer and the mixture incubated for an additional 90 min at 37°C. A volume of 2.5 ml ice-cold EDTA-buffer was added to stop the reaction and the degree of hemolysis measured. That dilution of serum required to form an average of one C6 site per cell (24) in this system was defined as the C6 titer.

Immune adherence reactions were performed in microtiter plates as follows. Serial dilutions of the patient's serum or control serum (25 μl in DVBS) were added to 25 μl DVBS and 50 μl of the complement-cell intermediate $EAC1_{GP}4_{Hu}2_{GP}$ (4.2×10^7 cells/ml). After 10 min at 37°C 25 μl human group O RBC ($OD_{541} = 1.3$) were added and the mixture incubated at 37°C. The plates were inspected at 15, 30, and 45 min for the appearance of an aggregated-cell settling pattern.

Serum bactericidal activity. This activity was studied with *S. typhi O 901* and *H. influenzae, type b.* Killing of *S. typhi* was measured by the turbidimetric growth assay of Muschel and Treffers (25) as modified by Rother, Rother, Peterson, Gemsa, and Mitze (26), with the following exceptions. The bacteria were taken from an 18-h broth culture in beef heart infusion; incubation of sensitized bacteria with human sera was at 37°C for 60 min. Specific rabbit antiserum to *S. typhi O 901* was kindly provided by Dr. Gabriel Michael, University of Cincinnati School of Medicine, Cincinnati, Ohio. In preliminary studies employing a 1/5 dilution of agammaglobulinemic human serum as the C source, it was found that maximum bacterial killing could be achieved over a broad range of dilutions of the rabbit antiserum from 1/100 to 1/12,800. A 1/6,400 dilution was chosen for the actual assays. Five normal sera and C6-deficient serum were tested concurrently as the C source, with and without the addition of 1/6,400 rabbit antiserum.

For bactericidal assays against *H. influenzae* 0.2 ml from an overnight broth culture was added to 5 ml fresh Levinthal's broth and incubated at 37°C for 4-h. This subculture was then diluted 1/1,000 in phosphate-buffered saline (PBS), pH 7.4, containing 3×10^{-4} M Ca^{++} and 2×10^{-3} M Mg^{++}. The reaction mixtures, in polystyrene tubes (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.), consisted of 0.025 ml of the diluted bacteria, 0.05 ml PBS, 0.05 ml of complement source (normal or patient's serum in twofold dilutions from undiluted to 1/32), and 0.025 ml immune rabbit serum, diluted 1/256 in PBS, to insure the presence of optimal antibody. Immediately after mixing (zero time) and after 60 min at 37°C the contents of each tube were streaked onto chocolate agar plates with calibrated loops. The plates were incubated at 37°C for 24 h in 5% CO_2, after which colonies were counted. Standard controls were run in each assay.

Chemotactic assays. Assays with peripheral white blood cells of normal human donors were performed in a modified Sykes-Moore chamber with a 3-μm Millipore filter, as described elsewhere (27). Results are expressed as a ratio, the chemotactic index, which is explained in reference 27 The formula used to calculate the chemotactic index in the present study is (28):

$$\frac{\text{number of cells (attractant side)}}{\text{number of cells (starting side)}} \times 500.$$

Escherichia coli O 111: B4. E. coli endotoxin (Difco Laboratories, Detroit, Mich.) or heat-aggregated (65°C, 20 min) human IgG served as activating agents. 4 vol of endotoxin (1 μg/ml) or aggregated IgG (1 mg/ml) were mixed with 1 vol of undiluted human serum serving as C source and allowed to stand for 20 min at room temperature before injection into the lower section of the chamber and the start of the 3-h incubation at 37°C. In all experiments normal and C6-deficient sera were tested concurrently, using the white blood cells of a given normal donor. To obtain the dose-response curves shown in Fig. 3 the standard concentration of activating agent was mixed (4 vol : 1 vol) with twofold dilutions of normal or C6-deficient serum in Hanks' solution, allowed to stand for 20 min, and

inserted into the chamber for the usual 3-h incubation at 37°C.

Complement binding to red cells by cold agglutinins. Group O adult RBC (2.5%) were incubated in 1/10 dilutions of fresh serum and isolated anti-I cold agglutinins at 20°C for 90 min. After a 10-min incubation at 37°C the RBC were washed 3 times in 37°C saline, resuspended to 2.5% and tested in antiglobulin (Coombs) reactions with anti-C3 and anti-C4 as previously described (17). The cold agglutinins used had previously been purified by adsorption to human RBC at 4°C and elution at 37°C.

Paroxysmal noctural hemoglobinuria (PNH). Lysis of PNH red cells employed a standard acid hemolysis test (29) and a modification of the sugar water test (30) as follows. Washed RBC were suspended to 50% in test serum. To 0.1 ml of this cell suspension was added 0.9 ml of the sucrose solution, and the mixture was incubated at 37°C 30 min. After centrifugation the degree of lysis in supernates (diluted 1/20) was determined by OD at 413 nm.

Cryoglobulins. Cryoglobulins were measured and isolated as previously described (31), using 10 ml serum separated at 37°C as starting material. After washing 3 times in cold PBS, pH 7.3, the cryoprotein was redissolved at 37°C in PBS (1/10 to 1/20 of the original serum volume). Immunodiffusion analysis of the redissolved cryoproteins was carried out in 1% agar in PBS. Initial incubation was at 37°C for 5 h and then overnight at room temperature. These studies employed appropriate reference proteins as positive controls, e.g., purified IgM, IgG, IgA, C3 or C4, or, in the case of anti-C6, normal human serum.

RESULTS

Initial studies. Despite the consistent lack of detectable hemolytic activity in CH_{50} titrations of fresh serum from patient D. B., C3 on two occasions was 107 and 170 mg/100 ml (normal range 70–220) and C4 was 34 mg/100 ml (normal range 20–40) by radial immunodiffusion. In a hemolytic assay employing a 4-ml C source (20) a mixture of 2 ml 1/200 D. B. serum with 2 ml 1/200 C2-deficient human serum produced 100% lysis of EA, whereas 4 ml of either diluted serum alone yielded < 5% lysis. Moreover, D. B. serum and normal serum, serially diluted, gave similar endpoints (1/8) in Ouchterlony precipitin reactions with rabbit antiserum to human C2.

TABLE I
Functional Assay of Complement Components

Component	Patient's serum (D. B.)	Reference serum
	U/ml	
C1	148,947	51,905
C4	218,126	141,000
C2	877	775
C3	3,636	4,000
C5	3,200	1,600
C6	0	47,500
C7	1,600	1,600
C8	64,000	64,000
C9	12,000	32,000
$\overline{C1}$ INH*	14.7 mg/100 ml	16.2 mg/100 ml
C3 proactivator‡: normal by immunoelectrophoresis		

* $\overline{C1}$ INH, C1 esterase inhibitor measured by radial immunodiffusion.

‡ C3 proactivator, properdin factor B or glycine-rich β-globulin.

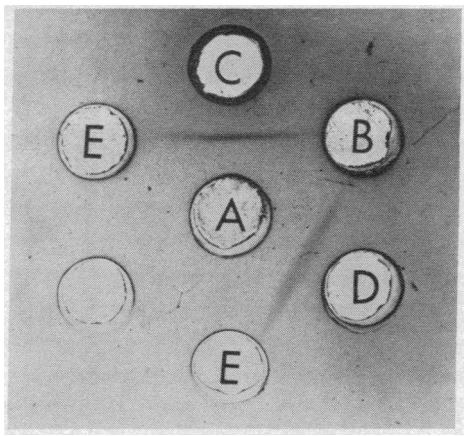

FIGURE 1 Two-dimensional gel diffusion reactions between (A) goat antihuman C6, (B) D. B. serum, (C) serum of father of D. B., (D) normal human serum, and (E) empty well (1% agarose in neutral Veronal-buffered saline; diffusion at room temperature, 20 h; stained with amido black).

As a functional test of early acting C components, advantage was taken of the ability of cold agglutinins to mediate sublytic binding of C components to human RBC in vitro in the presence of fresh serum (17, 32). Cell-bound C4 and C3 can be detected on such washed RBC by virtue of their agglutination by anti-C4 or anti-C3 sera (see Methods). When D. B. serum and normal human serum were compared as the C sources in such experiments they were equally effective in rendering the RBC agglutinable by both anti-C4 and anti-C3, implying functional activity of C1 through C3 in D. B. serum.

Addition of the patient's serum containing 0.005 M EDTA to the sensitized sheep RBC intermediate EAC-142 failed to cause lysis while EDTA-treated normal serum produced potent hemolysis. Thus, from these initial studies it appeared likely that D. B. serum had a late C component deficiency.

Detailed complement assays. Shown in Table I are results of functional assays for each of the C components in the serum of patient D. B. and in a normal serum titrated at the same time. It seems clear that the lack of whole hemolytic C activity in D. B. serum is attributable to the complete absence of C6. Other C components in D. B. serum were within normal limits. The absence

TABLE II

Test of C6 Inhibition by C6-Deficient Human Serum

Source of C6	Prior incubation with	C6 activity by hemolytic assay* (OD_{412})
Normal human serum	Buffer	0.475
Normal human serum	D. B. serum	0.439
Purified human C6	Buffer	0.287
Purified human C6	D. B. serum	0.130

* Method I (see text).

of C6, which was based on an assay involving stepwise buildup of C components on EA with functionally pure reagents (method I), was confirmed by an independent method utilizing C6-deficient rabbit serum as a reagent supplying all components except C6 (method II). D. B. plasma was also assayed for C6 activity and none was observed.

Ouchterlony analysis using both rabbit anti-rabbit C6 and monospecific goat antihuman C6 revealed no precipitin lines with D. B. serum (Fig. 1). Both antisera consistently produced single precipitin lines with normal sera. Study of serial dilutions of the normal sera suggested that 10–20% of the normal concentration of C6 protein could have been detected by this means. It may also be noted in Fig. 1 that the C6 precipitin lines formed by the normal or paternal serum show no curvature as these lines approach the well (B) containing D. B. serum.

Attempts were made to detect the presence of a C6 inhibitor in D. B. serum. Either D. B. serum (0.1 ml) or buffer (0.1 ml) was mixed with 0.1 ml normal serum or partially purified human C6. The mixtures were incubated for 15 min at 37°C. Appropriate dilutions were then prepared (normal serum, 1/20,000; purified C6, 1/2,000) and C6 assayed (method I). As demonstrated by the degrees of hemolysis obtained (Table II) there

TABLE III

Restoration of Hemolytic Activity in C6-Deficient Human Serum by Purified Human C6

Test mixture				Hemolytic activity (OD_{541})
1/60 D. B. serum	C6*	Buffer	EA‡	
ml	*ml*	*ml*	*ml*	
0.5	0.5	2.25	0.5	0.077, 0.063§
1.25	0.5	1.5	0.5	0.530, 0.516§
1.25	—	2.0	0.5	0.014

* Functionally pure human C6, 600 U/ml.

‡ EA (5×10^8 cells/ml).

§ Duplicate values.

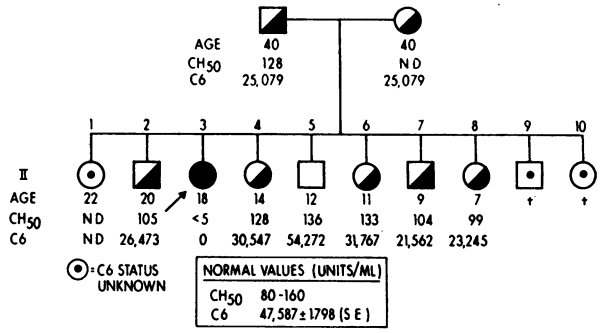

FIGURE 2 Family pedigree showing CH_{50} and C6 values for each family member. Solid black symbol, proposed homozygote; half-black symbols, proposed heterozygotes; open square, normal. Concerning deceased siblings, see Case Summary.

was only a minor reduction in the functional activity of normal serum C6. Although there was greater loss of activity with the partially purified C6, clearly hemolytic activity remained after the incubation. Since mammalian sera have been reported to possess an inhibitor of activated C6 (33), this loss of activity in the purified C6 preparation may reflect partial activation of C6 during purification. The appropriate control, i.e. normal human serum depleted of C6 by other means and then incubated with the purified C6, was not available. In any event, and most importantly, as shown in Table III, addition of functionally pure C6 to D. B. serum fully restored hemolytic activity. By $y/1-y$ plot (21) the CH_{50} of the reconstituted D. B. serum was 33 U/ml (normal sera >25 U/ml by this assay).

Cryoglobulins. The small quantity of cryoprecipitate described in the Case Summary was washed and redissolved in a minimum volume of PBS (1/10–1/20 of original serum volume). Immunodiffusion against anti-C3, anti-C4, and anti-C6 all gave negative results. Reference antigens, however, produced clear precipitin lines with each antiserum. Functional assay (method I) also failed to detect C6 in the redissolved cryoglobulin.

Family studies. Shown in Fig. 2 is the family pedigree, with age, whole hemolytic C (CH_{50}) titer and specific C6 titer (method I) listed for each family member studied. Using the same reagents, C6 assays were carried out on a panel of 14 normal controls,[2] from which the mean $\pm$SE are given in the box insert (Fig. 2). On repeated determinations both parents and five of the six available siblings had approximately half the normal level of C6. One sibling (II-5) had C6 values clearly in the normal range.

The sera of all family members, other than the proband, exhibited a clearly visible precipitin line in two-

[2] Controls comprised healthy NIH controls and staff, ages 18–40.

TABLE IV

Serum Bactericidal Activity Against S. typhi 0 901

Source of complement	Rabbit antibody added*	% killing with test serum (C source) diluted					
		Undil.	½	¼	⅛	1/16	1/32
C6 deficient	Yes	0	0	0	0	5	ND
C6 deficient	No	0	2	1	1	1	ND
Five normals‡	Yes	ND	ND	100±0.4	86±17	19±19	2±3
Five normals	No	ND	ND	98±1	56±30	4±2	5±0.8
C2 deficient	Yes	100	100	76	6	2	ND
C2 deficient	No	99	100	67	4	5	ND

* Specific antiserum to *S. typhi 0 901*, diluted 1/6,400.

‡ Mean±SD.

dimensional gel diffusion against anti-C6. Quantitative radial immunodiffusion with antirabbit C6 appeared capable of distinguishing normal sera from sera which had shown half-normal C6 functional levels. This antiserum was relatively weak, however, and the ring diameters were small, making readings difficult.

Also noteworthy in Fig. 2 is the finding that half-normal levels of C6 were sufficient to allow normal whole C (CH_{50}) titers. This indicates that the heterozygous C6-deficient state apparently cannot be detected by whole hemolytic C titration, presumably because even half-normal C6 levels are not limiting in such assays.

18 other normal individuals have had C6 measurements on other occasions. All but one had C6 titers in the normal range. One white female, however, has consistently shown C6 values slightly lower than the proposed heterozygotes in Fig. 2. This subject, therefore, may well be heterozygous for C6 deficiency, although family studies have not yet been done.

Functional properties of C6-deficient human serum. D. B. serum displayed a total lack of bactericidal activity against *S. typhi 0 901,* in the presence or absence of optimal amounts of specific rabbit antibody to this organism (Table IV). For comparison, C2-deficient human serum killed very effectively at lower dilutions but was not equal to our normal controls at higher dilutions. Against *H. influenzae, type b,* in the presence of optimal rabbit antibody, C6-deficient human serum again exhibited no detectable bactericidal effect (not shown). C2-deficient serum, however, demonstrated excellent killing.[3]

D. B. serum also was unable to mediate lysis of human PNH red cells in either the acidified serum test or the sugar water test (Table V). In contrast, C2-deficient human serum tested concurrently produced excellent lysis in the acid hemolysis test but none in the sugar water test.

D. B. serum and a normal reference serum were compared for the capacity to contribute C3b to sheep EAC142 cells, conferring on them immune adherence reactivity with human RBC used as indicator cells. D. B. serum had a normal titer of 400 U (reference serum: 300 U), consistent with a late component defect.

Generation of chemotactic activity for human white blood cells by D. B. serum in the presence of bacterial endotoxin or heat aggregated human IgG was normal in each of three studies, one of which is presented in Table VI. This screening assay employs human serum in a final dilution of 1/5. In an attempt to make the assay more sensitive to possible differences between normal and C6-deficient serum, increasing dilutions of normal and C6-deficient sera were tested as C sources in the presence of aggregated IgG or endotoxin. As

TABLE V

Lysis of PNH Red Cells in Complement-Deficient Sera

Test	Serum serving as C source	% lysis	
		Normal RBC	PNH RBC
Acid hemolysis	Normal, untreated	1.0	1.5
	Normal, pH 6.5	1.6	25.7
	Normal, pH 6. 5., inact.	ND	0.7
	C6 deficient, untreated	1.1	0.3
	C6 deficient, pH 6.5	1.7	0.6
	C6 deficient, pH 6.5, inact.	ND	0.3
	C2 deficient, untreated	1.1	1.5
	C2 deficient, pH 6.5	1.7	26.5
	C2 deficient, pH 6.5, inact.	ND	0.5
Sugar water	Normal	7.1	54.0
	C6 deficient	4.1	4.1
	C2 deficient	3.1	3.4

[3] The *Hemophilus* bactericidal assays were kindly performed by Mrs. Sheri Simon and Dr. Richard Robertson, Department of Microbiology, University of Rochester.

TABLE VI

Generation of Chemotactic Activity for Human White Blood Cells in C6-Deficient Serum

Source of complement	Activating agent	Chemotactic index*
D. B. serum	Endotoxin (1 μg/ml)	227‡
Four normal sera	Endotoxin (1 μg/ml)	188±44§
None (control)	Endotoxin (1 μg/ml)	68‡
D. B. serum	Aggregated IgG (1 mg/ml)	185‡
Four normal sera	Aggregated IgG (1 mg/ml)	131±15§
None (control)	Aggregated IgG (1 mg/ml)	41‡

* See Methods for definition.

‡ Mean of triplicate determinations.

§ Mean±SD for four normals. Each normal serum was tested in triplicate, concurrently with D. B. serum.

shown in Fig. 3, a dose-response curve was obtained but significant differences between C6-deficient and normal sera were not observed, even at limiting concentrations of serum.

DISCUSSION

Evidence has been presented for the first known occurrence of hereditary C6 deficiency in a human kindred. The proband, a young black female, was discovered because her serum lacked hemolytic C activity. Detailed functional analysis of her serum C components revealed selective absence of C6. C6 was also undetectable by immunodiffusion against specific antisera, thus providing evidence against the presence of a nonfunctional C6 protein in her serum.

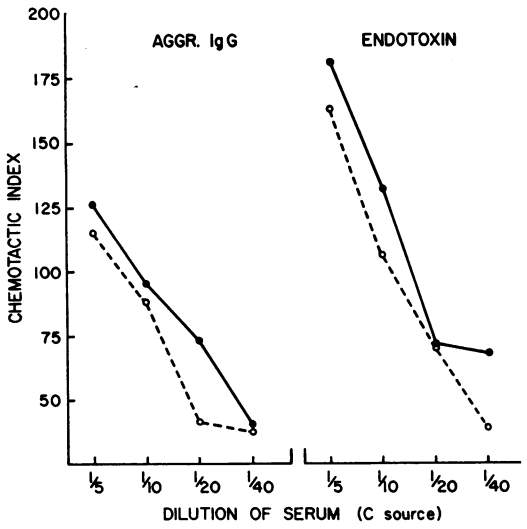

FIGURE 3 Effect of increasing dilution of normal and C6-deficient human serum on generation of chemotactic activity in the presence of constant concentrations of aggregated IgG (1 mg/ml) or endotoxin (1 μg/ml). Each point is the mean of triplicate determinations within the experiment. ●——●, C6 deficient; ○ - - - ○, normal.

That the absence of C6 represents a synthetic defect is suggested by (*a*) the selective nature of the C defect; (*b*) the genetic analysis (Fig. 2); (*c*) our inability to attribute the C6 defect to a serum inhibitor or to binding by the small quantity of cryoglobulins present in the proband's serum. The absence of C6 in her plasma rules against consumption of C6 during blood clotting (34–36).

The C6 deficiency appears to follow classic mendelian inheritance, with all three possible genotypes recognizable within this family (Fig. 2). A similar mode of inheritance has been observed in human C2 deficiency (3–6, 9) and C3 deficiency (12, 14). The heterozygous state in the present kindred can be recognized by specific C6 titration, with heterozygotes having about half-normal functional levels of C6 (Fig. 2). In this respect the inheritance of C6 deficiency in this family resembles that noted in C6-deficient rabbits (18, 37). The unexpected finding of an apparently healthy individual in the control population with a half-normal level of C6 raises the possibility that C6 deficiency in man may be less rare than expected.

C6-deficient rabbits have been the subject of detailed experimental analysis and it was, therefore, of great interest to compare the functional properties of the C6-deficient serum of the human proband. A detailed examination of hemostatic function is presented in the companion paper (16). The complete lack of bactericidal or cytolytic activity in the proband's serum is consistent with total absence of C6 activity. Similar findings have been reported in C6-deficient rabbits (26). The contrasting performances of C6-deficient and C2-deficient human sera in concurrent bactericidal assays against *S. typhi* (Table IV) and *H. influenzae* (see above) provide further evidence for a dominant role of the alternate pathway in this function (38–40).

C6-deficient rabbits apparently are not unusually susceptible to infections (1, 18) but are reported to be abnormally sensitive to endotoxin in vivo (41). Our homozygous C6-deficient patient has had no special problem with infections, including those due to gram negative bacteria, despite the total lack of serum bactericidal capacity. The latter mechanism may be less important in vivo than other mechanisms, e.g., intracellular killing. Possibly the patient has not had a maximal bacterial challenge, although she probably has had two episodes of gonococcemia without unusual complications.

C6-deficient rabbit serum originally had been reported to show deficient generation of chemotactic activity in

vitro in the presence of immune complexes (42). This defect seemed attributable to an inability to form the $C\overline{567}$ chemotactic complex (43), the only C-derived chemotactic factor known at that time. Subsequently, two other laboratories found that C6-deficient rabbit serum and normal rabbit serum produced equivalent amounts of chemotactic activity in response to endotoxin (44) or immune complexes (44, 45). Technical differences, particularly in pore size of filters, may have contributed to this discrepancy. In the present investigation, using filters similar to the later studies (44, 45), C6-deficient human serum supported normal generation of chemotactic activity in the presence of endotoxin or aggregated human IgG (Table VI and Fig. 3). The findings in Table VI were confirmed by Dr. John Gallin, National Institute of Allergy and Infectious Diseases, Bethesda, Md., using a different assay method (46). The generation of normal chemotactic activity in C6-deficient human serum appears to be attributable to adequate formation of C5a (45, 47, 48). A requirement for the $C\overline{567}$ complex was not evident under the conditions of our assay.

Also reported here is the observation that serum from this C6-deficient patient does not support lysis of PNH RBC at low ionic strength (sugar water test) or in acidified serum (Table V). This emphasizes the complement-dependent nature of PNH hemolysis. In contrast, C2-deficient human serum, shown to have an intact alternate pathway function but to totally lack an operative classical pathway, did support lysis in acidified serum but not in the sugar water test (Table V). These data support the conclusion that the lysis of PNH RBC proceeds via the alternate pathway in the acidified serum test (49–51), and via the classical pathway in the sugar water test. On the other hand, Logue, Rosse, and Adams (51) observed significant but subnormal lysis of PNH RBC incubated at low ionic strength with hereditary angioedema serum which had been incubated at 37°C immediately before testing in order to deplete residual C4. While some technical differences were involved, it is also possible that the hereditary angioedema serum was not as devoid of classical pathway activity as our C2-deficient serum.

As a final point, reference has already been made to the recent reports of rheumatic disorders and autoimmune phenomena in patients with hereditary C deficiences. The C6-deficient proband, patient D. B., is in good general health but the occurrence of mild Raynaud's phenomenon and "mixed type" cryoglobulins, however small in quantity, may be viewed with suspicion in an 18-yr-old female. Long-term follow-up will therefore be of great clinical interest.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Dr. Roger D. Owens for his assistance with logistic arrangements in the early phase of the study. Our thanks are also due to Patricia A. Thiem, Mary E. Kelly, and Charene Winney for able technical assistance, and to Jo Ann Burns for preparation of the manuscript.

The research was supported in part by U. S. Public Health Service grant AM-09810, the Pediatric Blood Research Fund, University of Rochester and by a U. S. Public Health Service General Research Support Grant awarded to the University of Rochester.

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