Hereditary Deficiency of the Seventh Component of Complement

J. T. Boyer, E. P. Gall, M. E. Norman, U. R. Nilsson, and T. S. Zimmerman

From the Section of Clinical Immunology, Department of Internal Medicine, College of Medicine, University of Arizona, Tucson, Arizona 85724; the Departments of Pediatrics and Medicine, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19174; and the Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California 92037

Abstract Deficiency of the seventh component of complement has been found in the serum of a 42-yr-old Caucasian woman who has Raynaud's phenomenon, sclerodactyly, and telangiectasia. Partial deficiency was found in the serum of the patient's parents and children, indicating a pattern of inheritance of autosomal codominance. Transfusion experiments indicated that exogenous C7 had a 91-h half-life in the patient. There was no evidence for C7 synthesis after transfusion. No C7 inhibitors were detected in the patient's serum. The patient's serum was found to support the activation of complement by both the classical and properdin pathways to the C7 stage. The addition of C7 to the patient's serum permitted it to support hemolytic reactions initiated by either pathway. No defects could be detected in plasma or whole blood coagulation. The patient's serum was deficient in opsonizing unsensitized yeast particles in serum and in the generation of chemotactic factor by antigen-antibody complexes and endotoxin. Both deficiencies were corrected by the addition of C7. These observations suggest a key role for C7 for in vitro yeast phagocytosis and chemotaxis generation. However, the patient's lack of infections indicates a relatively minor role for C7 in human resistance to infection.

This study was presented in part at the Second International Congress of Immunology, Brighton, England, 26 July 1974.

Dr. Gall was supported during part of this study by training grant TR-39 of the Veterans Administration. Dr. Nilsson is the recipient of National Institute of Allergy and Infectious Diseases Career Development Award 6K 04A-150198. Dr. Zimmerman is the recipient of Research Career Development Award K04H270242.

Received for publication 6 February 1975 and in revised form 30 May 1975.

INTRODUCTION

Discovery of genetic abnormalities of the complement system in man has resulted in the demonstration of the role of $C\bar{1}$ [1] inhibitor in preventing the generation of a permeability kinin, the role of C3 in the defense against bacterial infections, a relation between C1q deficiency and hypogammaglobulinemia, and a relation between inherited C5 abnormality and infection as well as the suspicion that deficiency in C1r or C2 predisposes the individual to a collagen disease (1). We report here the first case of hereditary C7 deficiency occurring in a woman with Raynaud's phenomenon, sclerodactyly, and telangiectasia. The patient's clinical course and experiments performed with the patient's serum offer new observations on the role of this component in man.

CASE SUMMARY

The patient, a 42-yr-old Caucasian woman of French and English descent, was originally seen for a diagnosis of Raynaud's phenomenon and early gangrene of the right index finger. Although she considered her past health to have been generally excellent, she reported that from late childhood she would awake with swelling and stiffness of the hands. She particularly noted swelling of the fingers after the use of a typewriter. In the area of swelling there would be considerable itching and pain, and she frequently noted that swollen areas were pink, tender, and warm. She discovered that she could get rapid relief of her symptoms by holding ice cubes to the swollen areas. All of these symptoms waned over the next several years,

[1] Nomenclature used here conforms with that agreed upon by the World Health Organization (*Bull. W. H. O.* **39**: 935–938, 1968) for the classical complement pathway and with the recommendations of the Complement Nomenclature Committee, Second International Congress of Immunology for the properdin pathway.

but in 1967 she first began to note that her hands and often her feet would blanch upon exposure to the cold with associated pain in affected areas. Gangrene of the terminal phalanx of the right index finger developed just before her original clinic visit in 1972. A number of asymptomatic, small red spots had appeared on the lips, face, and palms over the previous 5 or 10 yr. Her weight, appetite, and vigor had been constant and she was able to continue in a number of athletic activities. There was no consanguineous marriage within her family. Examination of her parents, her three brothers, and her two children failed to reveal any similar problems. She denied abnormal bleeding episodes, swallowing difficulties, change in bowel habits, shortness of breath, cough, chest pain, rash, drug sensitivity, loss of muscle strength, or tightening of the skin (except intermittently in her fingers associated with swelling).

On physical examination she appeared in good health. The skin was slightly redundant, wrinkled, and highly mobile over the face, neck, and arms. Punctate telangiectasia of 1–3 mm in diameter were present on the palms, face, lips, buccal mucosa, and nasal mucosa. These blanched slightly, but not completely on pressure. The forearms and hands showed soft tissue swelling that would pit slightly on pressure. Range of motion was normal except for slight loss of complete finger flexion due to the swelling. The skin of the hands showed no trophic changes and hair growth and skin thickness were normal. There was dry gangrene in a 1×2-cm area of the palmar aspect of the tip of the right index finger. Radial pulses were normal bilaterally, although ulnar pulses were absent. Dorsalis pedis and posterior tibial pulses were normal. Exposure of any extremity to mild cold or exposure of the patient to a cold room caused marked pallor and usually associated pain in the hands or feet. After blanching, there was no marked rebound or hyperemia, but a slow reversion to normal color.

Laboratory examinations. WBC 5,200; polymorphonuclear leukocytes (PMN)[2] 57; lymphocytes 37; monocytes 5; and eosinophils 1%. Hematocrit 41; sedimentation rate (Wintrobe) 18. Antinuclear factor negative. Rheumatoid factor negative. Cold agglutinin titer negative. Cryoglobulins negative. Creatinine 0.8 mg/100 ml. Calcium, phosphorus, alkaline phosphatase, and serum glutamic oxaloacetic transaminase normal. Barium swallow revealed no abnormal pattern. X rays of the extremities showed no soft tissue calcifications; however, there was ossification of the radial-ulnar interosseus membrane bilaterally. The chest X ray and upper gastrointestinal series were normal.

The patient was treated with intra-arterial injections of reserpine of both brachial and both femoral arteries with marked relief of her Raynaud's phenomena and rapid improvement in the gangrenous area of the finger.

2 yr after her first visit, the patient had elective facial cosmetic surgery. The surgical procedure was unremarkable except for a striking vasoconstrictive response of the cut edges of skin to injected epinephrine, there being almost no bleeding from the edges of the wound. The postoperative course was complicated by small areas of necrosis at the wound edge behind each ear; however, these healed very well with only slight scarring. Skin fragments were normal on histologic section except for the presence of mild solar elastosis.

During the 2 yr the patient has had several minor episodes of infections, all thought to be viral in nature, all resolving normally without treatment.

METHODS

Sera. Human serum was removed by venapuncture, allowed to clot and separate for 2 h at room temperature, and frozen at −85°C in aliquots. The study of C7 titers in a normal population was made on individual samples from blood bank donors ($n = 47$). "Normal" levels of the remaining components of complement were by analysis of pooled normal sera ($n = 25$). Human sera defective in opsonizing yeast particles were provided as before (2). Human C2d and C6d serum, deficient in the second and sixth components of complement, respectively, were kindly provided by Dr. John Leddy, University of Rochester School of Medicine and Dentistry, Rochester, N. Y. Sera from two patients with hereditary telangiectasia were generously provided by Dr. Richard R. Miller of Tucson, Ariz. C4-deficient guinea pig serum was obtained by cardiac puncture from rabbits supplied by Dr. Michael Frank, National Institute of Allergy and Infectious Diseases, Bethesda, Md. C6-deficient rabbit serum was taken by arterial puncture from the ear of rabbits supplied by Rancho de Conejo, La Jolla, Calif.

Erythrocytes and cellular intermediates. Sheep erythrocytes for hemolytic assays were obtained locally and maintained at 4°C in Alsever's solution. Cellular intermediates EAC4 (human) and EAC1–7 (human) were purchased from Cordis Laboratories, Miami, Fla. EAC1,4,^{oxy}2 and EAC1,4,^{oxy}2,3 were made as described elsewhere.[3] Normal human erythrocytes were taken from a single donor and stored as above. Erythrocytes from a patient with paroxysmal nocturnal hemoglobinuria (PNH) were kindly supplied by Dr. Russell Weisman, University Hospitals of Cleveland, Ohio.

Complement reagents and assays. Gelatin veronal buffer (GVB) was prepared daily (3). Equal volumes of 5% glucose (Mallinckrodt Chemical Works, St. Louis, Mo.) were added to GVB to prepare glucose-GVB (Gl-GVB). This reagent was utilized in all hemolytic assays of complement and complement components. EDTA and its various salts were obtained as Sequestrene from Geigy Chemical Corporation, Ardsley, N. Y., as Na_3-EDTA, Na_2Mg-EDTA, and Na_2Ca-EDTA and dissolved in GVB (from which Ca^{++} and Mg^{++} were omitted) for use. Rabbit antibody to sheep erythrocytes and purified complement components C1–C9 were purchased from the Cordis Laboratories or supplied by the National Institute of Allergy and Infectious Disease. C5 (4) and C7 (5) were also made as described previously. Both C5 and C7 gave single lines in agarose gel electrophoresis and were functionally pure with respect to other components of the classical complement pathway. CH_{50}, C1, C2, C7, C8, and C9 titrations were performed according to the procedures described by Cordis Laboratories, by using Cordis intermediates and components (human). Cordis C7 titers were utilized only for initial diagnostic studies. C6 and C4 titrations were made by utilizing rabbit C6d (6) and guinea pig C4d (7) sera. C3 and C5 were assayed as described elsewhere.

[2] *Abbreviations used in this paper:* CRST syndrome, calcinosis, Raynaud's phenomenon, sclerodactyly, and telangiectasia; EA, sensitized erythrocytes; Gl-GVB, glucose-GVB; GVB, gelatin Veronal buffer; HPF, high-power field; PMN, polymorphonuclear leukocyte; PNH, paroxysmal nocturnal hemoglobinuria.

[3] Boyer, J. T., and P. Wyde. 1975. Hypochlorite-induced alterations of human serum complement. Submitted for publication.

A new method for titration of C7 was developed by using selected serum samples from the patient: All serum samples showing CH_{50} titers of less than 5 U/ml were deemed satisfactory. A 0.1-ml volume of each dilution of unknown was added to assay tubes containing 0.2 ml of Gl-GVB, 0.1 ml of the appropriate C7-deficient serum reagent (diluted as indicated by preliminary titrations), and 0.1 ml of sensitized erythrocytes (EA, 1×10^8 cells/ml). All mixtures were incubated at 37°C for 120 min, 1 ml of cold EDTA saline was added, and the supernates were separated for analysis at OD_{412}. In this report, all results citing C7 titers refer to the assay utilizing the patient's serum.

Acid serum hemolysis. The hemolysis of PNH erythrocytes in acidified human serum was performed as described by Hinz (8). Serum was gently rotated in a flask at room temperature in 100% O_2 until pH reached 8.4. 0.15 N HCl was added to obtain the desired pH. Equal volumes of 0.15 N saline were added to other samples to maintain constant concentrations of serum in all samples. 0.1 vol $MgCl_2$, 0.2 M, was added to all sera to give 0.02 M Mg^{++}, the optimal concentration for acid serum lysis. Cordis C7 or saline was added finally to certain serum samples. pH did not change on addition of Cordis C7. PNH erythrocytes were adjusted to desired pH by repeated washing with buffer before adding to serum-buffer or serum-C7 mixtures. pH was recorded as the value obtained by microelectrode measurement at room temperature of the total incubation mixture just before 37°C incubation. pH changed during 37°C incubation less than 0.2 pH U.

Hemolysis and complement coating of human erythrocytes by cold agglutinins. Purified cold agglutinins were used to coat normal human erythrocytes with complement, hemolysis was assayed, and Coombs tests were performed as reported elsewhere (9).

Reactive hemolysis. Assays for "reactor" and "indicator" as well as assays for C7 were performed by hemolysis of erythrocytes in agarose as described by Thompson and Lachmann (10), except that PNH erythrocytes were substituted for sensitized sheep erythrocytes because of their increased sensitivity. Essentially, erythrocytes suspended in melted agarose are poured to a thickness of 2 mm on a glass slide and allowed to gel. Holes are punched in the agarose and filled with human serum or serum reagent. Curved lines of visible hemolysis form between certain sera in a manner analogous to precipitin lines in an Ouchterlony plate. A serum on the concave side of the line is said to be a "reactor" and has been shown to contain excess C5 and C6. One on the convex side is an "indicator" and has an excess of C7, as compared with C5 and/or C6. "Activated reactor" is a serum containing free and active $C\overline{56}$ due to previous exposure to zymosan (10).

Specific antisera to complement components. Anti-C3, anti-C4, anti-C5, and antiproperdin were made as described previously (9, 11). Anti-C7 was kindly provided by Dr. Carlos Arroyave, Scripps Clinic and Research Foundation, La Jolla, Calif. Anti-factor B (anti-GBG) was kindly provided by Dr. Chester Alper, Children's Hospital Medical Center, Boston, Mass. Immunoelectrophoresis was performed as described elsewhere.[3]

Coagulation studies. Partial thromboplastin time, prothrombin time, platelet count, and bleeding time were performed as described previously (12). Whole blood clotting time was also performed as previously described by using 12×75-mm style polypropylene (Falcon no. 2053, Falcon Plastics, Division of BioQuest, Oxnard, Calif. and clear plastic (Falcon no. 2052) tubes at 25°C (12). Prothrombin consumption in plastic tubes was determined as described previously (12) with the exception that a 2-h incubation period was used.

Yeast phagocytosis. Phagocytosis of unsensitized yeast particles by human neutrophils was performed as described by Miller and Nilsson (2).

Chemotaxis. Human neutrophil chemotaxis was studied by a modification of techniques described previously (13) using human albumin-rabbit anti-human albumin complex or *Escherichia coli* lipopolysaccharide endotoxin to activate complement. Results were expressed as the average number of PMN's per high-power field (HPF) after counting 10 consecutive fields. Duplicate samples were measured in all cases. The range of nine normals was 16.5–19.0 PMN's/HPF.

Metabolism of C7. Turnover of unlabeled C7 in the patient was studied by infusing 200 ml fresh normal plasma from a blood group-compatible, HAA-negative blood donor who had donated 14 times previously without inducing hepatitis in the recipients. Informed consent was first obtained. Infusion time required 35 min. Radioiodinated albumin (^{125}I) was given in a bolus at the end of the infusion. "Zero time" of the study corresponded to the end of the infusion. Samples of clotted blood and heparinized blood were taken periodically and analyzed for C7 hemolytic activity and radioactivity. The half-life of C7 was estimated from the final slope of the curve representing the log concentration of injected C7 remaining in the plasma plotted against time. 394,000 U of C7 were transfused, producing an increase in titer from 19 U/ml at preinfusion to 200 U/ml in the 15-min sample.

RESULTS

Establishment of C7 deficiency

Routine complement studies were performed on the patient as a part of an ongoing study of all patients with diseases thought to involve immunologic mechanisms. The first CH_{50} titer was 7 U/ml (normal, 69–119) while quantitative immunoprecipitin and immunoelectrophoretic values for C3, C4, C5, properdin, and factor B were normal. The presence of an inhibitor in the patient's serum was ruled out by demonstrating that incubation of as little as 1/10 vol of normal serum with the patient's serum increased with CH_{50} to normal levels. Varying quantities of the patient's serum caused no reduction in CH_{50} when incubated with normal serum. Small amounts of sera showing specific complement component deficiencies–C2d (human), C4d (guinea pig), C6d (rabbit), and C6d (human)–all restored complement activity to the patient's serum. Thus, it was concluded that each of these components was normal in the patient's serum.

The patients serum caused no more hemolysis of EAC1 than of EA, indicating that the deficiency was not in C1. Finally, addition of each of the nine classical components of complement in the functionally pure state to aliquots of the patient's serum showed marked augmentation of hemolysis only upon the addition of C7. Fig. 1 shows a dose-response curve for partially puri-

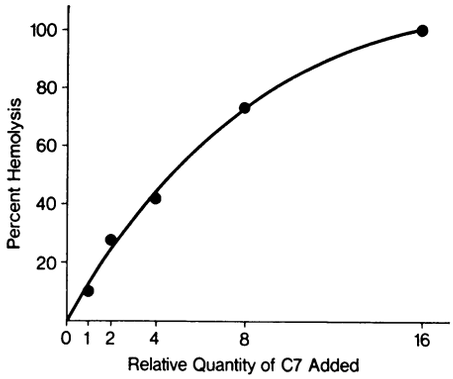

FIGURE 1 Dose-response curve of C7 vs. percent hemolysis of EA in the presence of the patient's whole serum.

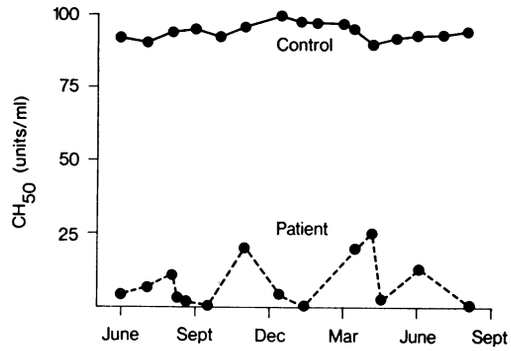

FIGURE 2 Variation in CH_{50} as a function of calendar time in the C7-deficient patient. Control serum taken from a single donor, not necessarily at the same time as from the patient.

fied C7 added to the patient's diluted serum. The curve is concave to the dose axis as predicted by the "one-hit hypothesis" (3).

Titers of the nine classical components of complement and immunoprecipitation of properdin and factor B are shown in Table I. The normal mean titer of C7 was 2,500, and 2 SD from the mean ranged from 1,785 to 3,337 U/ml. It is apparent that the deficiency of C7 in the patient, with values ranging from undetectable to approximately 3% of normal, accounts for the hemolytic deficiency of her whole serum. The remainder of her complement components are probably within normal limits, although precise normal limits have not been established in our laboratory. The variation in C7 titer or CH_{50} titer from different samples of blood could not be correlated with the state of the patient's health, her menstrual cycle, or the time of the year. Fig. 2 shows the variation in the patient's CH_{50} as compared to a normal individual over a 15-mo period. The lowest CH_{50} values corresponded to C7 titers of less than 10 U/ml. The highest CH_{50} titer was 26 U/ml and corresponded to a C7 titer of 75 U/ml. In the sera with the highest titers, faint precipitation lines against anti-C7 could be seen in Ouchterlony plates with reactions of identity between the antiserum and normal human serum. A serum sample having 3.5% of mean normal C7 level by hemolytic assay was found to have between 10 and 15% of normal C7 level by immunoassay (kindly performed by Dr. Carols Arroyave). This disparity implies that the patient's C7 is defective as well as deficient with respect to normals.

TABLE I
Complement Component Measurements

Component	C7-deficient patient	Normal pooled sera
	U/ml	
C1	51,000	81,000
C2	2,800	2,650
C3	18,500	19,000
C4	13,800	23,000
C5	1,800	3,000
C6	2,800	2,105
C7	<10–75	2,500
C8	820,000	360,000
C9	1,450,000	350,000
CH_{50}	0–32	95
Properdin	Normal by immunoprecipitation	
Factor B	Normal by immunoprecipitation	

Genetic studies

Fig. 3 compares the study of the patient's family to 47 normal donors and four patients showing similar telangiectasia Raynaud's phenomena. It is seen that the family members' C7 titers may be divided into two apparent normals, six ranging from 50 to 56% of normal, and one less than 3% of normal (the patient). The inheritance of C7 deficiency is also shown in Fig. 4 according to a hypothesis of simple Mendelian codominant inheritance. Both the patient's parents and both of her children are heterozygous, consistant with this hypothesis. Two brothers are completely normal while one is also heterozygous, a distribution also consistent with the hypothesis of Mendelian codominant inheritance. Only one other normal individual was found with C7 levels consistently below 2 SD from the mean of the C7 normals. Family studies have not been carried out in this individual, however. Although the patient's inherited abnormality was always detectable by CH_{50} determinations, the heterozygous state may only be determined by C7 titers. Two patients with hereditary telangiectasia had telangiectasia similar to the patient, but normal C7 levels. Two patients with calcinosis, Raynaud's phenomenon, sclerodactyly, and

telangiectasia (CRST syndrome) had C7 levels far in excess of any normal.

Studies of the functions of C7-deficient serum and plasma

Reactive hemolysis. The patient's serum showed "reactor" qualities, i.e., a concave line of hemolysis appeared in the agarose-containing PNH erythrocytes between a well containing the patient's serum and a well containing normal serum. This reaction has been shown to indicate that the patient's serum has an excess of complement components C5 and C6 over that of C7 (10). "Activated reactor," a source of $C\overline{56}$ (10), diffused through PNH erythrocytes containing agarose in the presence of sodium EDTA and failed to produce a line of hemolysis against the patient's serum, confirming the deficiency of C7 in the patient's serum. Finally, the titer of activated reactor from the patient's serum was compared with that generated in normal serum and was found to be almost 80 times increased. These data are summarized in Table II and are consistent with interpretations offered by Thompson and Lachmann (10): after fluid phase activation of the late-acting components of complement by agarose, C5, C6, and C7 react with one another to form a trimolecular complex. If the amount of C5 and C6 exceeds that of C7, the remaining $C\overline{56}$ is a highly reactive complex (activated reactor) which will combine with additionally added C7 and activate C8 and C9 to produce hemolysis. If C7 molecules are present in excess of C5 and C6 in a given serum, fluid phase activation of late-acting components of complement depletes all C5 and C6, but leaves normal C7 (indicator). This C7 is available for combination with $C\overline{56}$ in reactive hemolysis. Since our patient was markedly deficient in C7, her serum behaved as a "reactor" as expected. Further, there being so little C7 present, almost all $C\overline{56}$ remained after complement activation as "activated reactor" and yielded the highest titer of this complex in any serum measured to date.

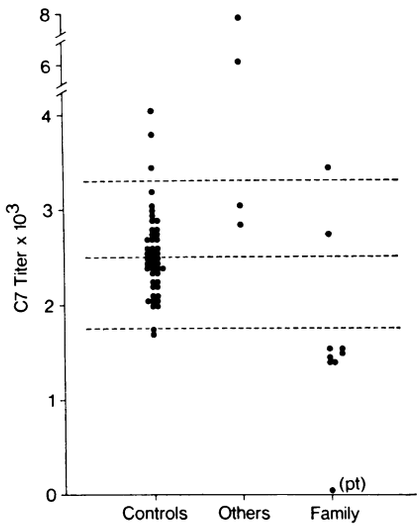

FIGURE 3 C7 titers of normal blood donors, four other patients with telangiectasia, and the patient's family. Among the four other patients, the two with the high values had CRST syndrome; with the two lower values, hereditary telangiectasia. The horizontal lines denote the mean and 2 SD.

TABLE II
Reactive Hemolysis

Reactions with:	Normal sera: Natural reactor	Normal sera: Natural indicator	Patient's serum
Natural reactor	0	+	0
Natural indicator	+	0	+
Activated reactor (C56)	0	+	0
Titer of activated reactor	1/64	0	1/5,000

Sera from normal donors known to be either natural reactors or natural indicators were used to induce hemolysis in agarose-containing PNH erythrocytes against each other or the patient's serum in incubations at 25°C overnight. Activated reactor serum was made by incubating serum of a natural reactor with zymosan at 37°C for 60 min (10).

Complement coating and hemolysis by cold agglutinins. Incubation of the patient's serum with ABO-compatible erythrocytes and purified cold agglutinins for 60 min at 25°C produced excellent coating of the cells with C3, C4, and C5 as indicated by marked agglutination of the washed cells with appropriate antisera. Normal serum gave the same results, but also produced traces of hemolysis. Addition of C7 to the patient's serum caused no discernable change in the reaction.

Acid serum hemolysis of PNH erythrocytes. Fig. 5 shows the degree of hemolysis of PNH erythrocytes under acid and alkaline conditions, with or without the addition of functionally pure C7. It is apparent that the patient's serum caused much less hemolysis of PNH erythrocytes than did normal serum, especially under acid conditions. This deficiency was almost totally corrected by the addition of C7. It is also apparent that C7 augmented the hemolytic effect of normal serum, indicating that C7 is probably limiting in normals as well as the patient with respect to acid serum hemolysis of PNH cells.

Opsonization of yeast particles. The patient's serum was compared with normal sera in its ability to promote phagocytosis of yeast particles by neutrophils. The results, shown in Table III, indicate that there was a deficiency with respect to the opsonification of normal yeast particles treated directly with her serum. The addition of C7 corrected this defect.

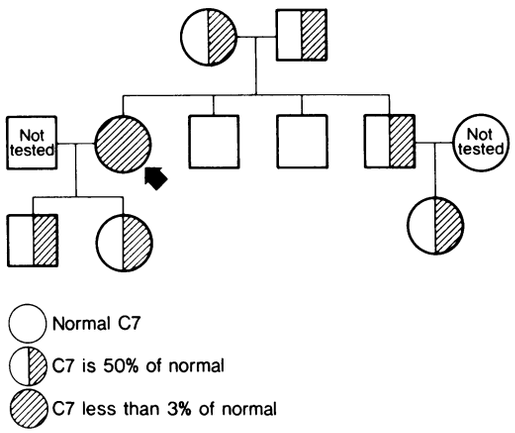

FIGURE 4 The inheritance of C7 deficiency. The arrow marks the proposita.

Chemotaxis. Fig 6 demonstrates that the patient's serum was less than half as potent as normal serum in generating chemotactic factors in response to antigen-antibody complexes. The addition of partially purified C7 partially restored this deficiency. In addition, it was shown that either normal serum or the serum of C6-deficient individuals restored activity of C7-deficient serum in the generation of chemotaxis.

Coagulation. No abnormalities of the hemostatic system could be detected. The partial thromboplastin time (patient: 29.4 s, control: 34.8 s); prothrombin time (patient: 11.1 s, control: 11.4 s); thrombin time (patient: 11.1 s, control: 13.3 s); platelet count (patient: 210,000 mm^3); and bleeding time (patient: 7 min, normal: < 10 min) were all within normal limits.

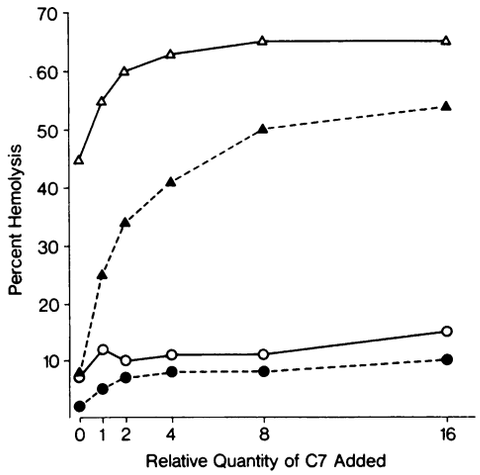

FIGURE 5 Acid serum hemolysis of PNH erythrocytes in normal (solid lines, open symbols) and the patient's serum (interrupted lines, closed symbols) at pH 8.4 (circles) and pH 6.7 (triangles) as a function of added C7.

TABLE III
Phagocytosis of Yeast

Yeast incubated with:	C7 added	Phagocytic index
	μl	
Normal serum	—	3.91
Normal serum	5	3.97
Buffer	—	1.37
Buffer	5	1.41
Patient's serum	—	2.65
Patient's serum	5	3.87

Yeast was incubated in normal or the patient's serum for 60 min at 37°C, washed, and added to neutrophil preparation. The average number of yeast particles ingested per neutrophil is the phagocytic index.

C6-deficient rabbits have been shown to have prolonged whole blood clotting times and deficient prothrombin consumption. These abnormalities were most obvious when the tests were carried out in plastic tubes (12). Accordingly, these tests were performed in a similar manner with blood from the propositus. No abnormalities could be detected. The whole blood clotting time in polypropylene tubes was 305 min (normal: 240–355 min, $n = 14$) and in clear plastic tubes 325 min (normal: 240–355 min, $n = 14$). Prothrombin consumption at the end of 2 h in either tube was 50% (normal: 40–70%, $n = 4$).

Metabolism of C7. Fig. 7 demonstrates the survival of C7 from transfused normal plasma in the patient. This is the first study of C7 catabolism reported in man or animals. An explanation for the irregular C7 levels during the early phases of equilibration is not apparent, but a half-life of 91 h was estimated from the final slope of the curve. The validity of this measurement is based upon the assumption that the patient's endogenous C7 production was stable during the period of study. Plasma

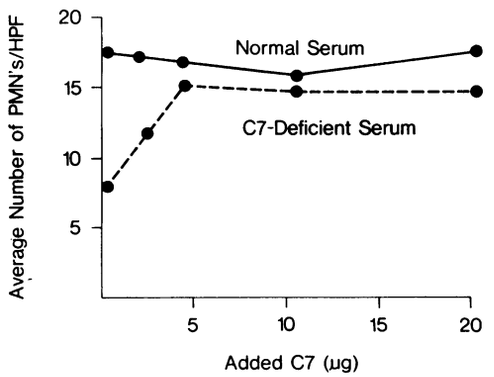

FIGURE 6 Effect of adding C7 to normal and C7-deficient serum on chemotaxis.

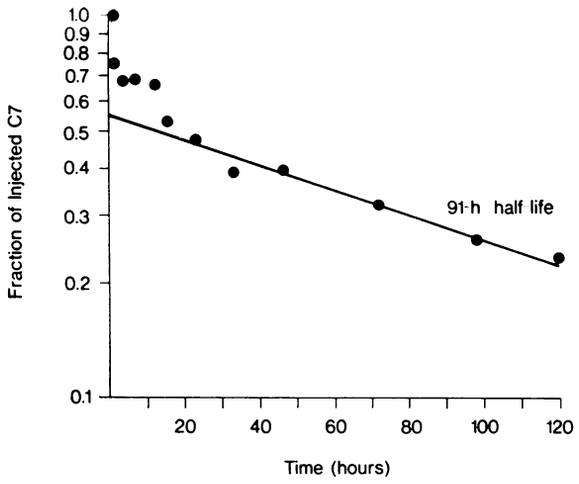

FIGURE 7 Survival of C7 of plasma transfused into the patient.

volume was calculated by radioiodinated serum albumin as 2.44 liters and by C7 concentration as 2.18 liters.

DISCUSSION

The defect in C7 in this patient appears to be caused by the inheritance of two autosomal alleles, each defective in coding for the synthesis of C7. The excess of C7 antigen over C7 function in the small amount of C7 present further indicates that the C7 molecule itself is defective. It is uncertain whether one parental mutant codes for zero C7 and the other for reduced and defective C7, or whether mutant genes are identical. The heterozygous family members range from 50% of normal C7, which might be presumed to be due to a combination of one normal gene plus one gene coding for zero C7, to 56%, perhaps representing a normal gene plus a defective one coding for 6% of normal C7. Assay accuracy, C7 fluctuation with time, and the wide variation in C7 level in normals make this interpretation hazardous, however. The C7-deficient patient of Wellek and Van Es, reported since the beginning of this study, had both zero C7 function and antigen (14). Both defective genes therefore coded for zero C7 antigen in their patient, and so the possibility exists that one of our patient's C7 genes does also. Whether the defective gene(s) involves synthesis of C7 at a much slower rate, whether the abnormal C7 molecule is catabolized at a more rapid rate, or both cannot be determined from our data.

The evidence that this inherited defect involves genes directly responsible for the synthesis of C7 is as follows: First, no inhibitors of whole serum complement or specific inhibitors of C7 were detected (an individual having an inhibitor of C7 has been reported previously [15]). Second, C7 antigen was also low to absent in the patient's serum. Though a functional defect in the C7 molecule accounted for a portion of C7 hemolytic deficiency, most of the latter was due to a deficiency in the concentration of C7 molecules in the serum. Third, the half-life of transfused, normal C7 was 91 h, a relatively long value compared to other complement components such as C3 and C4 (16); therefore, it is unlikely, though still possible, that hypercatabolism could account for the patient's C7 deficiency. Information on the C7 half-life in normal individuals is required to resolve this question. (Even if C7 half-life in normals should be determined to be 91 h, however, one could argue that the patient's defective C7 molcules might be catabolized faster than transfused normal C7.) Finally, the progressive fall in transfused C7 in the patient's blood indicates that no C7 was synthesized directly as a result of the transfusion. This suggests that the defect in production of C7 was not due to the absence of a precursor as is the case of factor VIII precursor in von Willebrand's disease (17).

It is apparent that the patient's deficiency is neither absolute nor constant. Whether C7 levels in normals or heterozygous individuals also varies with time, is not known. Fig. 2 shows marked fluctuations of the patient's CH_{50} which may be attributed to variations in C7, the limiting component in her serum. However, similar judgements of C7 level in normals may not be made from CH_{50} data because C7 is in such excess that variations of the magnitude of the C7 changes in the patient ($\pm 3\%$) would be undetectable. Only very precise C7 assays in normals over a period of time can provide this information.

Although the homozygous C7 deficiency is easily detected by routine CH_{50} measurement, detection of the heterozygous state requires direct C7 measurement, for the latter routinely has normal CH_{50} levels. Because the margin between low normal C7 levels and the heterozygate levels for C7 is so narrow, genetic studies are required for complete identification. Though there have been two individuals with homozygous C7 deficiency identified (14), it seems likely that this easily detected genetic defect is rare.

The use of the patient's serum to study various complement-dependent hemolytic systems confirms the role of C7 in both the classical and properdin pathways in the human. The addition of purified C7 to the patient's serum restored the hemolysis of EA. The coating of components C3, C4, and C5 on human erythrocytes by cold agglutinins is another manifestation of the classical complement pathway activity, but is expected even in the presence of C7 deficiency. Similarly, studies of reactive hemolysis and acid serum hemolysis of PNH erythrocytes confirms the role of C7 in hemolysis via the properdin pathway (8, 10). It is of interest that the

addition of C7 to normal serum considerably augmented acid serum hemolysis of PNH erythrocytes, indicating that C7 is limiting in this system. The addition of C7 to normal serum does not augment whole complement titers for EA. From our data, it is estimated that no more than 10% of the normal C7 concentration is needed for fully normal CH_{50} titers. This seems to indicate a relative efficiency of C7 in the classical pathway and inefficiency of C7 in the properdin pathway.

Two findings for the functions of the C7-deficient serum are unexpected: the defect in the phagocytosis of yeast particles and the defect in chemotaxis. It has been shown that C3 coating is responsible for the opsonizing of cells or particles sensitized with antibody which has activated the classical pathway of complement (18). Therefore it would be expected that serum deficient in any single complement component acting after C3 should support phagocytosis of antibody-coated particles, and such was found by Wellek and Van Es for the serum of their patient with C7 deficiency (14). In contrast, the phagocytosis of unsensitized bakers yeast in serum requires C3 (19), C5 (2), and, as shown in this report, C7. However, normal yeast phagocytosis occurring in a patient with neither the function nor the antigens of C5[4] (20) indicates that the requirements of the yeast phagocytic system are still to be determined.

Defective response to the generation of chemotactic factor in the patient's serum bears on the role of $\overline{C567}$ in chemotaxis. Three chemotactic factors, C3a, C5a, and $\overline{C567}$ have previously been identified as having chemotactic activity in various systems in vitro (16). The fact that the sera of C6-deficient rabbits and C6-deficient man are not defective in chemotaxis has been interpreted as evidence against an important role for $\overline{C567}$ in chemotaxis (21). However, purified $\overline{C567}$ has been shown to be strongly chemotactic, though $\overline{C56}$ was not (22), suggesting that C7 is an essential part of the trimolecular complex. Our data suggest that it is $\overline{C57}$ that may be of major importance to in vitro chemotactic systems of whole serum. Nilsson and Müller-Eberhard have shown that a natural affinity between C5 and C7 exists in free solution and that there is a stabilizing influence of C5 and C7 on EAC1,4,2a,3 (23). It seems likely that the affinity of C5 and C7 for each other would continue to be present upon activation and might possibly be augmented by that activation. Thus, generation of chemotactic factors in human serum by antigen-antibody complexes or endotoxin generates C3a, C5a, and $\overline{C567}$. In the C7-deficient serum of our patient, C3a and C5a are presumably formed normally. $\overline{C56}$ is also generated, but, as with the $\overline{C56}$ complex described by Lachmann and colleagues (22), is inactive in chemotaxis and fully effective chemotaxis is not obtained. In C6-deficient rabbits or man, a $\overline{C57}$ complex is presumably generated which has full chemotactic activity equal to the $\overline{C567}$ generated in normal serum. Obviously, the same arguments for the essential role of C7 that have been raised in the matter of chemotaxis may also be true for the explanation of the role of C7 is unsensitized yeast phagocytosis described above. In the case of opsonization, however, it is not clear why C7 is required for full effect when complement is activated by a "nonclassical" pathway and is not required when activated by the classical pathway, since C3 is required in each and membrane binding is essential. It may be that yeast particles are deficient or abnormal in C3 binding, revealing a larger role for the bound C7. Finally, any excitement generated by evidence for an essential role of C7 in chemotaxis and phagocytosis in vitro is mitigated greatly by the fact that our patient does not appear to suffer from infections, which would have been presumed to be the most likely associated clinical defect.

The blood coagulation parameters in this patient were within normal limits as were those of a previously reported patient with C6 deficiency (24). The findings in these two individuals contrast with the blood coagulation abnormalities previously observed in rabbits congenitally deficient in C6 (12). This difference probably relates to the difference in complement susceptibility exhibited by rabbit as opposed to human platelets. Whereas platelets lyse and develop markedly increased coagulant activity (platelet factor 3) after properdin pathway activation of complement with inulin, bacterial endotoxin, or cobra venom factor[5] (25), normal human platelets do not. In the rabbit these changes are dependent upon action of the terminal complement components (C5–9) and are associated with uptake of these proteins by rabbit platelet membranes. Such uptake does not occur with normal human platelets when complement is activated under similar conditions (26). Zymosan, after incubation in citrated plasma, does generate an activity which causes human platelets to release radiolabeled serotonin, and a properdin pathway activation of complement appears to be involved in the generation of this activity (26, 27). However, *terminal* complement components play little, if any, role in this reaction, and lysis does not occur (26).

While the patient's general health has been good, the question remains whether the combination of Raynaud's phenomenon, sclerodactyly, telangiectasia, and ossification of the radial-ulnar interosseus ligament are coincidental or related to the C7 deficiency. Her disease most resembles the CRST syndrome, yet C7 levels were high in the CRST patients studied. Collagen disorders have

[4] Nilsson, U. R. 1974. Unpublished observations.

[5] Zimmerman, T. S., and W. Kolb. 1975. Platelet membrane uptake of terminal complement components following activation of the alternate pathway with cobra venom. To be published.

been associated with genetic complement defects so frequently that it is tempting to include our patient among these; however, the individual deficient in C7 reported by Wellek and Van Es is completely free of disease (14). Until better evidence for a "collagen diathesis" in genetic complement deficiencies is obtained, it is probably best to consider this relationship coincidental.

ACKNOWLEDGMENTS

The authors wish to thank James Ambrose, Jane Tomb, and Judy Andrews for technical assistance and Margo Walter and Nancy Putignano for preparation of the manuscript.

This research was supported in part by U. S. Public Health Service grants AM13609, HL15491, AM13515, HL-15061, AI00319, AI00514, and AM13515 as well as a grant from the Hartford Foundation.

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