Inherited Deficiency of the Seventh Component of Complement Associated with Nephritis

PROPENSITY TO FORMATION OF $C\overline{56}$ AND RELATED C7-CONSUMING ACTIVITY

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ABSTRACT A 46-yr-old female with chronic pyelonephritis was found to lack complement (C) activity by the use of hemolytic screen assays in agarose gels. These assays also revealed a propensity of patient serum to form an activated complex of the fifth and sixth components of C, $C\overline{56}$. Each of the C component hemolytic activities was present in normal or elevated amounts with the exception of C7, which was undetectable; addition of purified C7 led to the restoration of hemolytic activity. C-dependent phagocytosis, immune adherence, and neutrophil chemotaxis were normal. Family studies demonstrated that the defect was transmitted as an autosomal codominant apparently not linked with alleles at the HLA-A or HLA-B loci. Persisting $C\overline{56}$ was readily formed in this as compared to normal serum upon incubation with multiple C activators including zymosan, inulin, immune complexes, heat-aggregated human gamma globulin, endotoxin, and agarose. A heat-stable (56°C, 30 min) activity which consumed C7 with time- and temperature-dependent kinetics was detected in plasma and serum, and seemed to be similar to a "C7 inactivator" previously described in another C7-deficient individual. However, this activity was found to have properties identical to those of $C\overline{56}$ during low ionic strength precipitation and chromatography on Sephadex G-200, to be specifically removed upon passage through an anti-C5 immunoadsorbent column, and to be associated with a small amount of $C\overline{56}$, suggesting that it represents an expression of small amounts of $C\overline{56}$ rather than a new C-inhibitory activity. Thus, an individual with chronic nephritis lacking C7 is reported; the utility of a hemolytic screen assay in agarose plates for the detection of such patients is emphasized; persisting $C\overline{56}$ is shown readily to be formed in this serum; and the presence of C7-consuming activity which is associated with and in all likelihood attributable to $C\overline{56}$ is shown.

INTRODUCTION

Genetic deficiencies of the terminal complement (C)[1] components of man are rare, but their discovery has greatly enhanced the understanding of the role of C in host defense. Patients with deficiencies of C3 (2–4) and C3b-INA (5, 6), as well as with dysfunction of C5 (7, 8), have an enhanced susceptibility to certain bacterial infections. In addition, patients with deficiencies of the C attack mechanism proteins C6 (9, 10), C7 (11), and C8 (12) also seem to have a propensity to infections, particularly with bacteria of the *Neisseria* groups.

Three patients with inherited C7 deficiency have been the subject of complete reports. One was a 42-yr-old woman with Raynaud's phenomenon, sclerodactyly, and telangiectasia (13); a second was a healthy 12-yr-old boy (14, 15); and a third was a 44-yr-old woman (with two healthy homozygous siblings) with ankylosing spondylitis (16). In addition, a family with three members lacking C7 has recently been presented in

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[1] *Abbreviations used in this paper*: BSA, bovine serum albumin, C, complement; EA, antibody-sensitized sheep erythrocytes; GGVB, GVB diluted with an equal volume of 5% glucose; GVB, veronal-buffered saline containing 0.1% gelatin; HLA, human leukocyte antigens; C7D, C7-deficient; C3B-INA, C3b-inactivator; $C\overline{567}$-INH, $C\overline{567}$-inhibitors.

which two of the deficient individuals suffered meningococcal meningitis in childhood and disseminated gonococcal infections as adults (11), and we have recently observed a 28-yr-old woman with deficiency of C7 and systemic lupus erythematosus.[2]

In this report, we describe an additional patient with deficiency of C7 who presented with a history of recurrent urinary tract infections and end-stage renal disease, and emphasize the usefulness of hemolytic assays in agarose gels for the detection of this deficiency. In addition, we report both a propensity to $C\overline{56}$ formation and the presence of an activity inhibitory to C7 in the patient's serum with evidence indicating that these phenomena are related.

CASE REPORT

C. A. was a 46-yr-old black woman who was hospitalized after 4 days of severe low back pain which had occurred intermittently for 1 yr. She was known to have had hypertension with suspected renal disease and repeated urinary tract infections for approximately 7 yr, although no prior work-up had been undertaken. She denied recent dysuria, frequency, urgency, or changes in the color or quantity of urine. Both parents (now deceased) had renal disease and hypertension, and her four children (ages 17–25 yr) are alive and well.

The patient was alert, well-developed, and obese on admission, with a blood pressure of 160–130/130-100 mm Hg. Her physical examination was remarkable only for oculovascular changes characteristic of hypertension. The hemogram was normal, the erythrocyte sedimentation rate (Westergren) was 58 mm/h, and the C-reactive protein level was 16 μg/ml. Bone marrow biopsy showed a hypocellular marrow which otherwise was normal.

The BUN level was 68 mg/ml (normal, 7–22 mg/ml) and the serum creatinine was 3.8 mg/ml (normal, 0.1–1.3 mg/ml). Urinalysis was normal except for 14 neutrophils and 1–3 erythrocytes/high power field on microscopic examination; no casts were seen. A 24-h urine specimen contained 6.5 mg of total protein (normal, 2–150 mg) and decreased electrolytes. The creatinine clearance was 26 mg/min per m^2 (normal, 80–110 mg/min per m^2). Urine cultures contained *Proteus mirabilis* (40,000 organisms/ml); multiple specimens of both urine and sputum were negative for acid-fast bacteria. The serum uric acid level was 11.5 mg/dl (normal, 3–6 mg/dl); inorganic phosphorous, 5.3 mg/dl (normal, 2.5–4.5 mg/dl; and vitamin B12, 87 pg/ml (normal, 300–1,000 pg/ml). The prothrombin time was 75% normal. Other blood chemistry evaluations were in or near the normal range.

The serum protein electrophoresis and immunoglobulin levels were essentially normal. Thymus-derived (T) lymphocyte (84%, 2070/mm^3) and bone marrow-derived (B) lymphocyte (4%, 99/mm^3) levels also were normal, as were the proliferative responses of patient cells to both phytohemagglutinin (stimulated, 74,690 cpm; background, 320 cpm/min) and mitomycin-treated homologous lymphoid cells (test, 26,323 cpm; control, 320 cpm). Anti-cardiolipin, rheumatoid factor, and antinuclear antibody assays were negative. Hemolytic complement activity was undetectable; description of the complement abnormality is outlined in detail below.

Radiograms showed skeletal changes interpreted as those of hyperparathyroidism secondary to renal failure. The intravenous pyelogram showed end-stage kidneys of decreased size bilaterally (left, 10.5 cm; right, 9.5 cm) which concentrated the contrast media poorly.

The discharge diagnosis was chronic renal failure attributable to chronic pyelonephritis, hypertension, and secondary hyperparathyroidism.

METHODS

Reagents. EDTA and zymosan (lot 24C-0941-9) were purchased from Sigma Chemical Co. (St. Louis, Mo.). Endotoxic lipopolysaccharide from *Escherichia coli* 0127:B8 (lot 25491) was purchased from Difco Laboratories (Detroit, Mich.). Inulin was kindly provided by Dr. Tibor Borsos of the National Institutes of Health. Sepharose 4B (used as the agarose source), Protein A-Sepharose CL-4B and Sephadex G-200 superfine were purchased from Pharmacia Fine Chemicals Inc. (Uppsala, Sweden). Bovine serum albumin (BSA)—anti-BSA complexes and aggregated human gamma globulin was prepared as previously described (17).

Buffers. Veronal-buffered saline, pH 7.2, containing 0.1% gelatin (GVB) was prepared as previously described (17). GVB was made to 10 mM in EDTA (GVB-EDTA), or 1 mM in $MgCl_2$ (GVB^+). GVB diluted with an equal volume of 5% glucose and brought to 1mM $MgCl_2$ and 0.15 mM $CaCl_2$ ($GGVB^{++}$) was prepared as described (18).

Erythrocytes. Unsensitized and sensitized sheep erythrocytes (E and EA, respectively), as well as guinea pig erythrocytes were prepared and stored as previously described (19).

Sera and serum components. Functionally pure human C3, C5, C6, C7, C8, and C9, as well as a reagent containing the C components C2–C7, were obtained from the Cordis Laboratories, Inc. (Miami, Fla.). Human $C\overline{56}$ was partially purified from human reactor serum as previously described (17). Normal human sera obtained from healthy laboratory personnel, C6-deficient rabbit serum, and sera from patient C. A. and her family were separated and stored at −70°C immediately after collection. Antiserum to human C5 prepared in goats was purchased from Meloy Laboratories Inc. (Springfield, Va).

Complement assays. Protein levels of Clq, C4, C3, C5, properdin, and properdin factor B were estimated by radial immunodiffusion by established methods as previously described (20). Total C hemolytic activity and assays for the hemolytic activities of each of the C1–9 components except C7, were performed as described (10, 20). C7 first was assayed using purified C components. $EAC\overline{14}$ (1×10^7), prepared according to Rapp and Borsos (21), and having a Tmax of 5 min, were incubated with 20 U each of C2, C3, C5, and C6, and serial dilutions of test serum in 0.1 ml $GGVB^{++}$ for

[2] Zeitz, H., G. Miller, T. F. Lint, A. Ali, and H. Gewurz. 1978. Systemic lupus erythematosus with deficiency of the seventh component of complement and solubilization of immune complexes in C-deficient sera. Manuscript in preparation.

30 min at 30°C (total volume, 0.3 ml); 0.1 ml containing 15 U of both C8 and C9 in $GGVB^{++}$ was added, and incubation was continued for 60 min at 37°C. The reaction was stopped by the addition of 1.0 ml of cold GVB-EDTA, the unlysed cells were removed by centrifugation, the absorbance of the supernate was determined at 412 nm, and the $C7H_{50}$ was calculated. In later experiments, after the selective deficiency of C7 in the patient's serum had been established, C7 also was assayed using the serum of patient C. A. In this assay, $EAC\overline{1423}$ (1×10^7), prepared according to Lachmann et al. (22), were incubated with a 0.1-ml $1/150$ dilution of patient C. A. serum and 0.1-ml serial dilutions of test serum in $GGVB^{++}$ in a total volume of 0.3 ml for 90 min at 37°C. Lysis was quantitated and calculated as usual. The C7-consuming activity in C. A. serum (see below) did not interfere in this assay since the reactants were mixed in the cold (0°C) and C. A. serum was diluted two-fold beyond the level at which the activity inhibitory to C7 was detected during incubations at 37°C. Both assays yielded similar results when C7 activity was titered in either normal or half-normal (C7-deficient family members) sera.

Other assays. HLA typing was performed by microcytotoxicity assay as described (23). Yeast phagocytosis was quantitated according to Miller and Nilsson (7); immune adherence C activity was measured according to the assay of Nishioka (24); and generation of activity chemotactic for neutrophils was generously assayed by Dr. John P. Leddy (Rochester, N. Y.) according to the method of Rosenfeld et al. (25).

Hemolytic assays in gel. Screen plates containing EA were used for qualitative measurement of classical C-pathway hemolytic activity, and were prepared as described (10, 26). Briefly, 1.0-ml 3% agarose (L'Industrie Biologique Francaise, Gennevilliers, France) dissolved in GVB^+ and cooled to 60°C, was mixed with 2.5-ml of GVB^+ containing 1×10^9 EA. This mixture was poured into a 3.5-ml immunodiffusion plate (Hyland Diagnostics Div., Costa Mesa, Calif.), and after solidification, 2.1-mm diameter wells were prepared and filled with 5 μl of the test sample. The plates were incubated for 1 h at 37°C, overnight at room temperature, and examined for lysis by inspection.

Plates containing E in the presence of 0.01 M EDTA were used to quantitate the generation of $C\overline{56}$. These "reactor plates" were prepared by the addition of 1 ml 10^9 E in GVB-EDTA to a tube containing 1 ml 3% agarose (warmed to 60°C), 1 ml GVB-EDTA, and 0.5 ml guinea pig serum diluted $1/50$ in GVB-EDTA (17). The mixture was rapidly poured into a 3.5-ml immunodiffusion plate, wells were prepared and filled with 5 μl of test samples, and the plates were incubated as described above.

$C\overline{56}$ generation in normal, reactor, and C7-deficient serum. Normal, reactor, and C7-deficient sera were tested for the presence of $C\overline{56}$ after incubation with various C activators. 0.2-ml Sera and the activators were incubated for 60 min at 37°C, particulate material (inulin, agarose, and zymosan) was removed by centrifugation, and 5 μl of each mixture was applied to reactor plates for determination of the amount of $C\overline{56}$ generated. A standard curve was prepared using partially purified $C\overline{56}$ to allow the quantitation of the $C\overline{56}$ generated in the different sera.

Assays for reconstitution of hemolytic activity and for C7 consuming activity. Reconstitution of hemolytic activity was demonstrated using the second of the C7 assays described above. Various amounts of purified C7 (1 vol) were added to a $1/30$ dilution of 1 vol of C. A. serum and lysis of $EAC\overline{1423}$ was determined after 90 min at 37°C. To test for C7-consuming activity, varying amounts of C7 were incubated with C. A. serum for varying lengths of time before the addition of $EAC\overline{1423}$. Incubations were continued for an additional 90 min at 37°C and lysis was determined in the usual way.

Gel filtration of C. A. serum on Sephadex G-200. A 2.6 × 64-cm glass column was packed with Sephadex G-200 superfine and equilibrated with 0.1 M tris buffer, pH 7.5, which contained 0.09 M NaCl and 0.01 M EDTA. 5 ml of C. A. serum was applied and chromatographed at a rate of 7.3 ml/h and the A_{280} and C7-consuming activity were determined in each 5-ml fraction, the latter by incubation of 50 μl of each fraction with 50 μl of purified C7 (1 U/tube) for 60 min at 37°C; residual C7 hemolytic activity was determined by the subsequent addition of 0.1 ml of each $EAC\overline{1423}$ (10^7/tube) and C. A. serum (diluted $1/150$), incubation at 37°C for 90 min, and quantitation of lysis in the usual way. The elution profiles of internal IgM, C3, C6, and albumin markers were determined by standard hemolytic or protein assays, while highly purified $C\overline{56}$ was passaged through the column under identical conditions to determine its elution volume. Fractions with C7-consuming activity were pooled, concentrated by ultrafiltration on an Amicon PM-30 membrane (Amicon Corp., Scientific Sys. Div., Lexington, Mass.), and dialyzed against 0.01 M phosphase buffer, pH 7.5. Both the precipitate and supernate obtained by this procedure were assayed for $C\overline{56}$ as described by Baker et al. (27).

Adsorption of C7-deficient serum by anti-C5. 5 ml of Protein A-Sepharose CL-4B was mixed with 2 ml goat anti-human C5 (73 mg protein), rocked gently for 17 h at 4°C, poured into a 1.0 × 20-cm glass column, and washed extensively with 0.1 M phosphate buffer, pH 7.4. 0.2 ml of C7-deficient serum was applied to the immunoadsorbent, and the proteins which eluted with 0.1 M phosphate were pooled, concentrated to the original serum volume, and assayed for hemolytic C3 and C5 activities (10, 22), as well as for C7-consuming activity as described above. Albumin, IgM, C3, and C5 protein concentrations were measured by radial immunodiffusion (20). In separate experiments, both $C\overline{56}$ and the partially purified C7-consuming activity were passed through identical anti-C5 immunoadsorbent columns and assayed for residual activity as just described.

RESULTS

The detection of abnormal serum by hemolytic assay in agarose gels. C. A. serum was found to totally lack hemolytic C activity. It was of interest that this C deficiency came to attention when a hemolytic C screen assay involving lysis of sensitized sheep erythrocytes embedded in agarose was performed, and the unusual pattern shown in Fig. 1 was observed. No primary lysis was seen around the well containing the patient's serum, although intense lines of "reactive lysis" concave to the patient's serum appeared within 24 h between the wells containing the patient and normal sera, respectively. These lines are indicative of the formation of an activated complex of C5 and C6 ($C\overline{56}$), known to occur in sera which contain C5 and C6 in excess of C7 upon interaction with the agarose contained in the indicator system (26). The unusual pattern of this intense reactive lysis line in absence of a zone of primary lysis prompted further investigation of C. A. serum.

The complement profile in C. A. serum. Hemolytic C activity was lacking in fresh plasma as well as in

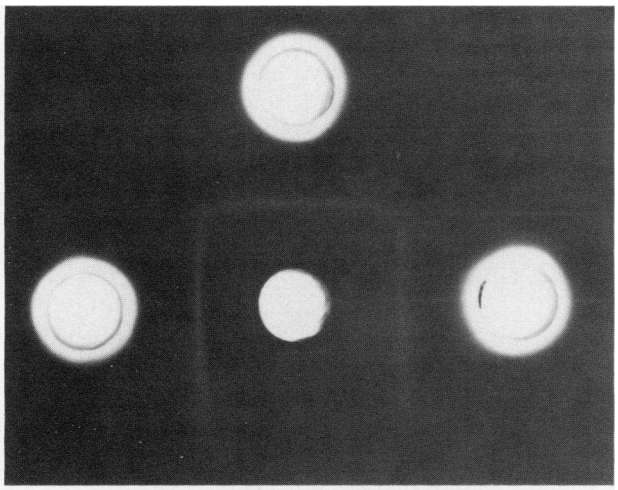

FIGURE 1 The demonstration of the inability of patient serum (center well), in comparison to normal sera (surrounding wells), to induce primary lysis of sensitized sheep erythrocytes suspended in agarose gels; however, $C\overline{56}$ was readily induced in patient serum as shown by the prominent lines of reactive lysis which formed between patient and normal sera.

serum, and this was found to be attributable to a selective and apparently complete absence of C7 (Table I). C component hemolytic activities of C1–9 with the exception of C7 were present in normal or elevated amounts, and hemolytic activity was reconstructed by small amounts of highly purified C7 (Fig. 2). The protein levels of C components Clq, C4, C3, C5, properdin, and properdin factor B were all present in normal or elevated amounts (Table I), whereas C7 antigen was undetectable (<3 μg/ml; normal, 60 μg/ml) (28). The C-dependent functions of phagocytosis of yeast particles and immune adherence, which require no C component acting later than C3, as well as the generation of neutrophil chemotactic activity, were normal in patient serum.

Inheritance of the C7 deficiency and its relationship to the HLA locus. Family studies were performed in order to define the genetics of the deficiency and its relationship to the inheritance of the HLA-A and HLA-B antigens. Normal levels of C7 established in a group of healthy adults showed a mean of 102,000 ±23,000 U/ml, with a range of 73,000–140,000 U/ml. As shown in Fig. 3, both children in whom C7 levels were measured had approximately one-half-normal values (40%–47%), three half-siblings had levels 30% normal, and a half-sister had an essentially normal (81%) C7 level. This pattern was consistent with autosomal codominant inheritance of the deficiency.

Only the Aw24 and B12 antigens were recognized at the HLA-A and HLA-B loci of the patient. However, another family member with this haplotype showed normal levels of C7, and two of the five family members with one-half normal levels of C7 lacked this haplotype. Thus, despite the detection of a single haplotype at the HLA-A and HLA-B loci, it would seem that the genes coding for the C7 protein in this family, as in the family reported by Delage et al. (16), were inherited independently of those coding for the HLA antigens, but additional studies are needed to confirm this interpretation.

Propensity to $C\overline{56}$ generation in C. A. serum. Persisting $C\overline{56}$ can be generated in the serum of certain individuals undergoing inflammatory reactions upon incubation with various activators of the alternative pathway, at least in part because of a relative excess of C5 and C6 over C7 (29). On this basis, the C7-deficient serum was expected to be particularly rich in its ability to develop $C\overline{56}$ upon activation of the alternative C pathway, and this proved to be the case. Large amounts of $C\overline{56}$ were readily generated upon incubation of C. A. serum with even small amounts of activators of either the primary (aggregated human gamma globulin, immune complexes) or the alternative (inulin, agarose, zymosan, lipopolysaccharide) C pathways (Table II). Indeed, the alternative pathway activators generated much more $C\overline{56}$ in C7-deficient serum than in reactor serum, whereas amounts of primary pathway activators which readily generated $C\overline{56}$ in the C7-deficient serum failed to generate $C\overline{56}$ in the reactor serum at all. None of the activators were able to generate $C\overline{56}$ in normal serum (Table II). Thus, the ease with which $C\overline{56}$ could be generated in C7-deficient serum was striking.

Demonstration and preliminary description of the C7-consuming activity in C. A. serum. C. A. serum was tested for C7-consuming activity, in part because

TABLE I
C-Component Profile

Hemolytic C activity	C1	C4	C2	C3	C5	C6	C7	C8	C9
Normal, %	262	129	116	128	119	91	<1	220	183

C Protein μg/ml	Clq	C4	C3	C5	Properdin	Factor B
Patient	245	780	1,130	120	21	266
Normal	110–210	200–800	800–1,800	70–170	10–20	175–225

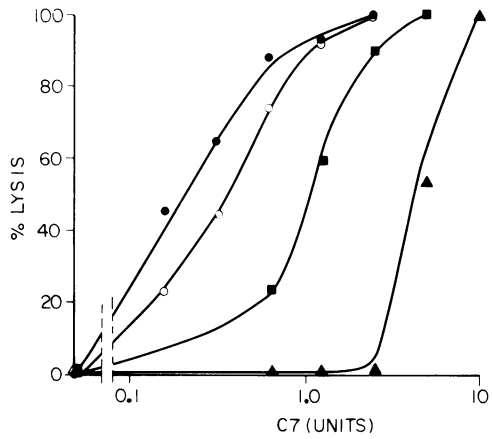

FIGURE 2 The reconstitution of hemolytic activity in patient C. A. serum (0.1 ml diluted 1:30 in GGVB^{++}) upon addition of small amounts of purified human C7 (0.1 ml) followed by incubation with EAC$\overline{1423}$ (1×10^7) in a final volume of 0.3 ml; the reactions were stopped by addition of 0.5-ml chilled GVB-EDTA, and the percentage of cells lysed was determined as described (●). In certain experiments, C. A. serum was preincubated with varying amounts of C7 for 5 (○), 15 (■), and 30 (▲) min at 37°C before the addition of the indicator cells.

a "C7-inactivator" had been described in a previously reported patient with C7 deficiency (15). Incubation of a small amount (10 U) of C7 (either as the highly purified component or as diluted normal human serum) with C. A. serum (1/5) for 30 min at 37°C resulted in the complete loss of C7 activity. When various amounts of purified C7 were preincubated at 37°C for 0–30 min with a 1/30 dilution of C. A. serum and residual C7 activity measured in the usual way, the results shown in Fig. 2 were observed. With increasing preincubation times, increasing amounts of C7 were required to achieve equivalent degrees of lysis. C7 incubated (30 min, 37°C) in buffer showed activity identical to that of the unpreincubated sample.

Preincubations (up to 60 min) in C. A. serum at 0°, 17°, or 23°C did not result in a detectable loss of C7 activity and depletion of C7 was seen only at 37°C. The C7-consuming activity was present in serum and plasma, and was unaffected by heating at 56°C for 30 min; it could be detected even at a 1/80 dilution of serum or plasma when limiting amounts of C7 (1–2 U) were used. It was active in the presence of 0.01 M EDTA, indicating that ongoing complement activation was not required for the activity to be expressed.

Demonstration of C$\overline{56}$ in C. A. serum. Since C$\overline{56}$ is readily generated in C. A. serum (Table II) and has C7-consuming activity in the experimental systems used (17), it seemed important to determine whether the inhibitory activity observed in C. A. serum was related to the presence of C$\overline{56}$. This was first attempted by passage of C. A. serum through a Sephadex G-200 column. As shown in Fig. 4, the C7-consuming activity eluted in a position identical to that of authentic C$\overline{56}$ passaged through the column under identical conditions. The C7-consuming activity in C. A. plasma also eluted in this position (data not shown).

To determine whether the fractions which had C7-consuming activity also contained C$\overline{56}$, aliquots were pooled, concentrated on an Amicon PM-30 membrane, dialyzed against 0.01 M phosphate buffer, and assayed for C$\overline{56}$ activity as described by Baker et al.

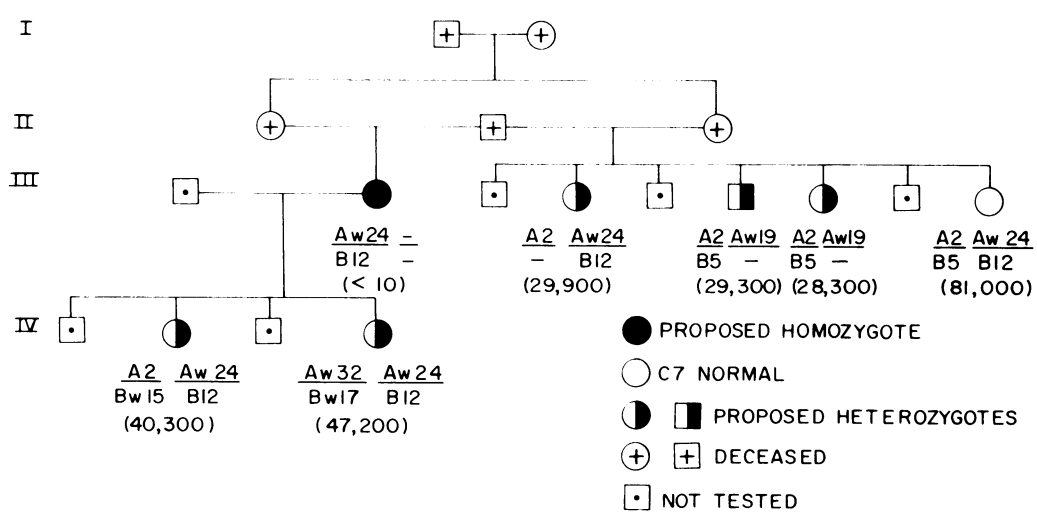

FIGURE 3 The pedigree of patient C. A. with HLA-A and HLA-B genotypes as well as C7 levels (in parentheses) indicated; the mean±SD $C7H_{50}$ was 102,000±23,000 (range, 73,000–140,000).

TABLE II
*$\overline{C56}$ Generation in C7-Deficient Serum**

Activating agent	$\overline{C56}$ generated		
	Normal serum	Reactor serum	C7-deficient serum
		U/ml	
None	<20	<20	<20
Aggregated human gamma globulin (8.3 mg/ml)	<20	<20	1,030
Ag-Ab‡ (8 μg/ml)	<20	<20	1,330
Ag-Ab‡ (80 μg/ml)	<20	<20	3,760
Ag-Ab‡ (800 μg/ml)	<20	860	4,250
Inulin (4 mg/ml)	<20	1,230	3,400
Agarose (36 mg/ml)	<20	1,320	3,550
Zymosan (2 mg/ml)	<20	2,250	5,270
LPS (5 mg/ml)	<20	2,300	6,800

* 0.2-ml sera were incubated with activators for 60 min at 37°C, particulate reactants were removed by centrifugation, and 5 μl of each mixture was applied to the appropriate plates for determination of the amount of $\overline{C56}$ generated. The diameters of the lytic zones were compared to those obtained with dilutions of a purified $\overline{C56}$ preparation of known hemolytic activity to establish unitage.

‡ Washed complexes of bovine serum albumin and rabbit anti-bovine serum albumin prepared at equivalence.

(17). The pooled fractions which showed C7-consuming activity also were found to contain significant amounts of $\overline{C56}$. This was unlikely to have been generated from C5 and C6 during dialysis against the low ionic strength buffer, since the fractions with C7-consuming activity contained no detectable C6. Thus, C. A. serum and plasma contained C7-consuming activity which eluted identically to $\overline{C56}$ on gel filtration and was associated with $\overline{C56}$ activity in sensitive hemolytic assays.

Adsorption of C7-consuming activity with anti-C5. To further explore the possibility that the C7-consuming activity was related to $\overline{C56}$, C7-deficient serum was chromatographed over an immunoadsorbent column that was prepared with anti-C5 bound to Protein A-Sepharose CL-4B. More than 94% of the native C5 and 76% of the C7-consuming activity in whole serum was removed by this procedure; by contrast, no (<5%) C3, (<10%) albumin, or (<10%) IgM assayed as controls were removed by the immunoabsorbant (Table III). In separate experiments, identical anti-C5 immunoadsorbants removed >92% of the purified $\overline{C56}$ and 84% of partially purified C7-consuming activity when equivalent amounts of protein were passaged through the columns. Although passage of unfractionated or partially purified C7-deficient serum through the immunoadsorbent did not result in loss of all of the C7-consuming activity, it is significant that the majority of the activity was removed despite the presence of a vast excess of native C5 which would be expected to compete for sites on the column. Thus, these

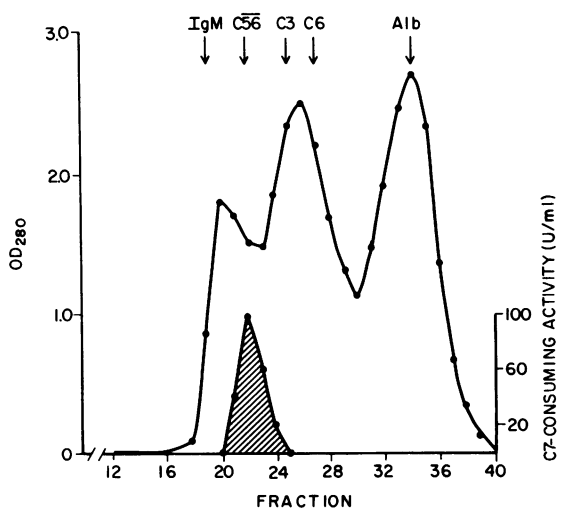

FIGURE 4 The application of C. A. serum (5.0 ml in 0.01 M EDTA) to Sephadex G-200 (2.6 × 64 cm) in tris (0.01 M)-EDTA (0.01 M)-NaCl (0.09 M) buffer (pH, 7.5) at 7.3 ml/h. 5.0-ml fractions were collected and the A_{280} (●) and C-7-consuming activity (●, striped area) were determined. The elution volumes of internal IgM, C3, C6, and albumin markers, as well as of subsequently applied $\overline{C56}$, are indicated.

TABLE III
*Adsorption of C7-Consuming Activity by Anti-C5**

Protein/activity applied	Protein/activity removed
	%
C5	>94
$\overline{C56}$‡	>92
C7-consuming activity	
Unfractionated	76
Partially purified§	84
C3	<5
IgM	<10
Albumin	<10

* C7-deficient serum C. A. was passed through an immunoadsorbent column consisting of goat anti-human C5 coupled to Protein A-Sepharose CL-4B in the presence of 0.1 M phosphate; the eluate was concentrated to the original serum volume and assayed for hemolytic C3 and C5, as well as for C7-consuming activities. Albumin and IgM protein concentrations were measured by radial immunodiffusion.

‡ Partially purified $\overline{C56}$ and C7-consuming activities, respectively, were passed through identical anti-C5 immunoadsorbent columns in separate experiments.

§ The C7-consuming activity was partially purified by chromatography on Sephadex G-200.

experiments indicated that the majority if not all of the C7-consuming activity was due to the presence of $C\overline{56}$.

C7-consuming activity in sera from other C7-deficient individuals. Sera from two unrelated C7-deficient individuals, J. A. (13) and G. S.,[2] were tested for the presence of C7-consuming activity using purified C7 as described above. No C7 activity or protein was demonstrable in J. A. serum, but serum from G. S. contained a small amount (0.2% normal) of hemolytically active C7. As shown in Fig. 5, J. A. serum displayed a degree of C7-consuming activity similar to that seen in C. A. serum; by contrast, only minimal C7-consuming activity was seen in serum from G. S. Since even small amounts of C7 would react with and neutralize small amounts of $C\overline{56}$ present in the C7D sera, these results are consistent with the concept that the C7-consuming activity is related to $C\overline{56}$. This generalization is in keeping with earlier observations that C7-consuming activity of C. A. serum was detected only when limiting amounts of C7 were used in the assay procedure.

DISCUSSION

This paper describes a C7-deficient individual with recurrent urinary tract infections and chronic renal disease. The absence of C7 was inferred to result from a genetic defect in C7 synthesis because of half normal C7 levels in two children and several half-siblings. All of the previously reported genetic deficiencies of C components have shown autosomal codominant inheritance, and the findings in this family are consistent with the same interpretation. Although only a single haplotype was detected at the major histocompatibility locus, raising the possibility of homozygosity for HLA as well as C7D, family studies suggested that the gene coding for synthesis of C7 protein was inherited independently of those coding for the HLA antigens. Since complete family studies could not be obtained, the data presented does not exclude the possibility of a distant linkage between HLA and C7 on chromosome 6, because one recombinant event could account for the single family member studied (the proband's half-sister III-9) with the HLA haplotype Aw24/B12 and normal levels of C7. However, evidence presented in the investigations of two other C7-deficient individuals (16, 29) clearly showed a lack of linkage between HLA and C7, as do recent studies on C7 polymorphism in man (30).

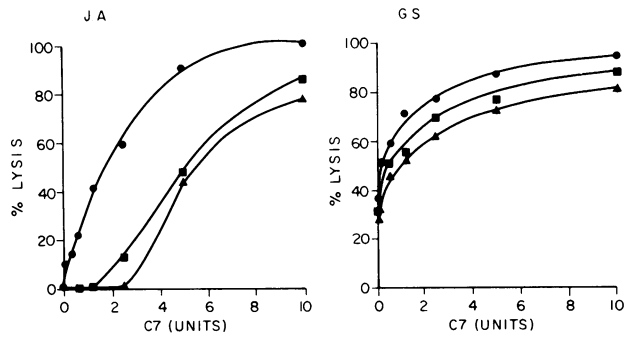

FIGURE 5 Reconstitution of hemolytic activity in C7D J. A. and G. S. sera (0.1 ml diluted 1:30 in $GGVB^{++}$) by small amounts of purified human C7 (0.1 ml). Samples were incubated with $EAC\overline{1423}$ (1×10^7) in a 0.3-ml final volume; the reaction was stopped by the addition of 0.5 ml chilled GVB-EDTA and the percentage of cells lysed was determined as described (●). In certain experiments the deficient sera were preincubated with varying amounts of C7 for 5 (■) or 15 (▲) min at 37°C before the addition of the indicator cells.

C7 deficiency was first reported in an individual with sclerodactyly and telangiectasia (13), and then later in an apparently normal adolescent (14), a 44-yr-old lady with ankylosing spondylitis (16), in several members of a family who have shown an unusual predisposition to gonococcal infections (11), and in a 28-yr-old lady (G. S.) with systemic lupus erythematosus. Although there is no proof that the C7 deficiency was causally related to the symptoms observed in these individuals or in the patient described above, the present experience does extend the group of diseases with which patients with deficiency of C7 and thus, of the terminal complement components present. The clinical manifestations associated with this deficiency seem to be quite variable, and are consistent with the concept that the deficiency of C7, like deficiencies of the early acting C components, predispose to certain infectious and autoimmune processes.

A striking feature of the deficient serum was its propensity to form persisting $C\overline{56}$ upon incubation with a variety of C activators, i.e. to show the properties of a "reactor serum" (26). Lachmann and Thompson (31) had proposed that the ability of acute phase sera to generate $C\overline{56}$ results from a relative excess of C5 and C6 over C7, based on the finding of reduced levels of C7 in reactor sera and an ability of normal serum to acquire reactor activity upon immuno-absorption with anti-C7. Although others have shown that $C\overline{56}$ can be generated via the primary pathway using a C5-consuming intermediate and purified C5 and C6 (32, 33), Baker et al. (17) have reported that, for reasons not yet clear, activators of the alternative but not the primary pathway readily induce persisting $C\overline{56}$ in acute phase sera. Nevertheless, substantial amounts of persisting $C\overline{56}$ were generated upon the incubation of C. A. serum with activators of the primary C pathway as well as with activators of the alternative C pathway. This would be expected should only minimal formation of $C\overline{56}$ occur via activation of the primary pathway, since the presence of C7 would abrogate the detection of $C\overline{56}$ by the simultaneous formation and immediate decay of $C\overline{567}$; only in serum markedly deficient in C7 would such $C\overline{56}$ be expected

to persist, and this seems to have been the case in the patient presented herein. The propensity of the deficient serum to form $C\overline{56}$ was reflected in the unusual pattern of lines of reactive lysis seen in absence of rings of primary lysis observed in the hemolytic reactions in agarose gels described above. This experience emphasizes the usefulness of assays in gel for detection of patients with deficiency of C7. We anticipate that such semiquantitative screening assays, because of their ease of performance, general accessability, and demonstration of unusual morphologic features such as those described above, will serve as useful adjuncts to the quantitative hemolytic tube and protein assays, and will help to uncover patients with abnormalities of the complement system generally (20, 34).

Initial studies of the deficient serum revealed an activity which consumed limited amounts of purified C7 as well as C7 in diluted serum or plasma; this occurred with time- and temperature-dependent kinetics and in the presence of EDTA, and persisted after heating at 56°C for 30 min. The propensity to form $C\overline{56}$ in C. A. serum prompted further characterization of the C7-consuming activity with special attention to its relationship to $C\overline{56}$, and four separate lines of experiments suggested that this activity was related to $C\overline{56}$: (*a*), passage of the deficient serum through Sephadex G-200 showed that the C7-consuming activity co-chromatographed with purified $C\overline{56}$; (*b*), fractions containing C7-consuming activity were found to contain significant amounts of hemolytically active $C\overline{56}$; (*c*), substantial amounts of the C7-consuming activity in diluted serum and in partially purified form could be adsorbed with anti-C5; and (*d*) the C7-consuming activity was observed only in C7-deficient sera totally lacking C7. Taken together, these results indicate that the majority, if not all, of the C7-consuming activity present in C. A. serum is due to persisting $C\overline{56}$. We cannot exclude the possibility that the C7-consuming activity may, at least in part, be attributable to a modified form of $C\overline{56}$ which retains the ability to bind C7 (35).

Wellek and Opferkuch (15) previously described a "C7-inactivator" in the serum of a C7-deficient individual which, although it had properties similar to many of those of the consuming activity found in the serum of our patient, was inferred to be distinct from $C\overline{56}$. This was based upon its migration on Sephadex G-200, the lack of associated $C\overline{56}$ activity, and an ability to inactivate C7 on a EAC1-7 intermediate. An activity with this latter property also was reported in two of four normal sera tested. These properties were assumed to derive from the same molecule and were offered as evidence for a new C-inhibitory principle termed the C7-inactivator. We do not know the basis for the difference between the results presented herein and the earlier studies of Wellek and Opferkuch. These authors did not (*a*) compare directly the elution profiles of purified $C\overline{56}$ and their inhibitory principle on Sephadex G-200; (*b*) take into account the effects of inhibitors of reactive lysis ($C\overline{567}$-INH) (17) which would markedly influence assays involving $C\overline{56}$ and C7; or (*c*) consider additional factors such as the incompatibility of human and guinea pig terminal C component activities (36). Based on the present studies, we would infer that the activity which they described might have been a manifestation of $C\overline{56}$ and perhaps in certain assays also of $C\overline{567}$-INH and (or) species incompatibilities and may not have represented a new C7 inhibitor. The reported absence of C7-inhibitory activity in C7-deficient serum by others (13, 16) probably is attributable to the degree of the deficiency (as cited above) and (or) to the type of C-consuming assay used, since both reports only involved assays for anti-total hemolytic C activities rather than for specific anti-C7 activity. Thus, whereas no inhibitory activity was demonstrated in C7-deficient serum J. A. in the initial report of this patient (13), such activity was readily demonstrated in his serum by use of the specific C7 inhibition assay employed in the present study.

The studies presented herein thus reveal that C7-deficient serum has an unusually great propensity for the generation of persisting $C\overline{56}$ upon incubation with activators of either the primary or the alternative C pathway. This is reflected in a distinctive pattern of lysis in gels which can be utilized for the rapid detection of C7 deficiency, and in the appearance of C7-consuming activity in serum and plasma which is associated with the presence and (or) ready formation of small amounts of $C\overline{56}$. The biological advantage, if any, of this propensity to form persisting $C\overline{56}$ is not yet clear.

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REFERENCES

1. Lint, T. F., S. G. Osofsky, G. R. Nemerow, K. Tausk, and H. Gewurz. 1976. Inherited deficiency of the seventh component of complement (C7) in man: description, relationship to HLA and partial characterization of an inhibitory activity. *Clin. Res.* **24:** 543. (Abstr.)
2. Alper, C. A., H. R. Colten, F. S. Rosen, A. R. Rabson, G. M. MacNab, and J. S. S. Gear. 1972. Homozygous deficiency of C3 in a patient with repeated infections. *Lancet.* **II:** 1179–1181.
3. Ballow, M., J. E. Shira, L. Harden, S. Y. Yang, and N. K. Day. 1975. Complete absence of the third component of complement in man. *J. Clin. Invest.* **56:** 703–710.

4. Alper, C. A., H. R. Colten, J. S. S. Gear, A. R. Rabson, and F. S. Rosen. 1976. Homozygous human C3 deficiency. The role of C3 in antibody production, Cls-induced vasopermeability and cobra venom-induced passive hemolysis. *J. Clin. Invest.* **57:** 222–229.
5. Abramson, N., C. A. Alper, P. J. Lachmann, F. S. Rosen, and J. H. Jandl. 1971. Deficiency of C3 inactivator in man. *J. Immunol.* **107:** 19–27.
6. Thompson, R. A., and P. J. Lachmann. 1977. A second case of human C3b inhibitor (KAF) deficiency. *Clin. Exp. Immunol.* **27:** 23–29.
7. Miller, M. E., and U. R. Nilsson. 1970. A familial deficiency of the phagocytosis enhancing activity of serum related to a dysfunction of the fifth component of complement (C5). *N. Engl. J. Med.* **282:** 354–358.
8. Nilsson, U. R., M. E. Miller, and S. Wyman. 1974. A functional abnormality of the fifth component of complement (C5) from human serum of individuals with a familial opsonic defect. *J. Immunol.* **112:** 1164–1176.
9. Leddy, J. P., M. M. Frank, T. Gaither, J. Baum, and M. R. Klemperer. 1974. Hereditary deficiency of the sixth component of complement in man. I. Immunochemical, biologic, and family studies. *J. Clin. Invest.* **53:** 544–553.
10. Lim, D., A. Gewurz, T. F. Lint, M. Ghaze, B. Sepheri, and H. Gewurz. 1976. Absence of the sixth component of complement in a patient with repeated episodes of meningococcal meningitis. *J. Pediatr.* **89:** 42–47.
11. Lee, T. J., P. D. Utsinger, W. J. Yount, and P. F. Sparling. 1977. Deficiency of the seventh component of complement associated with recurrent Neisseria infections. *Clin. Res.* **25:** 379. (Abstr.)
12. Petersen, B. H., J. A. Graham, and G. F. Brooks. 1976. Human deficiency of the eighth component of complement. The requirement of C8 for serum *Neisseria gonorrhoeae* bactericidal activity. *J. Clin. Invest.* **57:** 283–290.
13. Boyer, J. T., E. P. Gall, M. E. Norman, U. R. Nilsson, and T. S. Zimmerman. 1975. Hereditary deficiency of the seventh component of complement. *J. Clin. Invest.* **56:** 905–913.
14. Hannema, A. J., K. W. Pondman, U. Dohmann, H. Gadner, and L. J. Dooren. 1975. C7 deficiency in man. *Protides Biol. Fluids Proc. Colloq. Bruges.* **22:** 581–584.
15. Wellek, B., and W. Opferkuch. 1975. A case of deficiency of the seventh component of complement in man. Biological properties of a C7-deficient serum and description of a C7-inactivating principle. *Clin. Exp. Immunol.* **19:** 223–235.
16. Delage, J. M., P. Bergeron, J. Simard, G. Lehner-Netsch, and E. Prochazka. 1977. Hereditary C7 deficiency. Diagnosis and HLA studies in a French-Canadian family. *J. Clin. Invest.* **60:** 1061–1069.
17. Baker, P. J., L. G. Rubin, T. F. Lint, B. C. McLeod, and H. Gewurz. 1975. Binding of the complement intermediate $C\overline{56}$ to zymosan in acute phase human sera. *Clin. Exp. Immunol.* **20:** 113–124.
18. Nelson, R. A., J. Jensen, I. Gigli, and N. Tamura. 1966. Methods for the separation, purification and measurement of nine components of hemolytic complement in guinea pig serum. *Immunochemistry.* **3:** 111–135.
19. Mayer, M. M. 1961. Complement and complement fixation. *In* Experimental Immunochemistry. E. A. Kabat and M. M. Mayer, editors. Charles C Thomas, Publisher, Springfield, Ill. 2nd edition. 113–240.
20. Gewurz, H., and L. A. Suyehira. 1976. Complement. *In* Manual of Clinical Immunology. N. R. Rose and H. Friedman, editors. American Society for Microbiology, Washington, D. C. 36–47.
21. Rapp, H. J., and T. Borsos. 1970. Reagents commonly used in complement research. *In* Molecular Basis of Complement Action. Appleton-Century-Crofts, New York. 75–110.
22. Lachmann, P. J., M. J. Hobart, and W. P. Aston. 1973. Complement. *In* Handbook of Experimental Immunology. D. M. Weiss, editor. Blackwell Scientific Publications Ltd., Oxford. 2nd edition. 5.1–5.17.
23. Mittal, K. K., M. R. Mickey, D. P. Singal, and P. I. Terasaki. 1968. Serotyping for homotransplantation. XVIII. Refinement of micro-droplet lymphocyte cytotoxicity test. *Transplantation (Baltimore).* **6:** 913–927.
24. Nishioka, K. 1963. Measurements of complement by agglutination of human erythrocytes reacting in immune-adherence. *J. Immunol.* **90:** 86–97.
25. Rosenfeld, S. I., J. Baum, R. T. Steigbigel, and J. P. Leddy. 1976. Hereditary deficiency of the fifth component of complement in man. II. Biological properties of C5-deficient human serum. *J. Clin. Invest.* **57:** 1635–1643.
26. Thompson, R. A., and D. S. Rowe. 1968. Reactive haemolysis—A distinctive form of red cell lysis. *Immunology.* **14:** 745–762.
27. Baker, P. J., T. F. Lint, R. F. Mortensen, and H. Gewurz. 1977. $C\overline{567}$-initiated cytolysis of lymphoid cells: description of the phenomenon and studies on its control by $C\overline{567}$ inhibitors. *J. Immunol.* **118:** 198–202.
28. Müller-Eberhard, H. J. 1976: The serum complement system. *In* Textbook of Immunopathology. P. A. Miecher and H. J. Müller-Eberhard, editors. Grune and Stratton, Inc., New York. 45–73.
29. Rittner, C., W. Opferkuch, B. Wellek, H. Grosse-Wilde, and P. Wernet. 1976. Lack of linkage between gene(s) controlling the synthesis of the seventh component of complement and the HLA region on chromosome No. 6 in man. *Hum. Genet.* **34:** 137–142.
30. Lachmann, P. J., and M. J. Hobart. 1978. C6–C7: a further "complement supergene". *J. Immunol.* (Abstr.) In press.
31. Lachmann, P. J., and R. A. Thompson. 1970. Reactive lysis: the complement-mediated lysis of unsensitized cells. II. The characterization of activated reactor as $C\overline{56}$ and the participation of C8 and C9. *J. Exp. Med.* **131:** 643–657.
32. Goldman, J. N., and K. F. Austen. 1974. Reaction mechanisms of nascent $C\overline{567}$ (reactive lysis). II. Killing of a rough form of Escherichia coli by $C\overline{567}$, C8, and C9. *J. Infect. Dis.* **129:** 444–450.
33. Goldlust, M. B., H. S. Shin, C. H. Hammer, and M. M. Mayer. 1974. Studies of complement complex C5b,6 eluted from EAC-6: reaction of C5b,6 and EAC4b3b, and evidence on the role of C2a and C3b in the activation of C5. *J. Immunol.* **113:** 998–1007.
34. Gewurz, A., T. F. Lint, J. L. Roberts, H. Zeitz, and H. Gewurz. 1978. Homozygous C2 deficiency with fulminant lupus erythematosus: severe nephritis via the alternative complement pathway. *Arthritis. Rheum.* **21:** 28–36.
35. Yamamoto, K., and H. Gewurz. 1978. The complex of C5b and C6: isolation, characterization, and identification of a modified form of C5b consisting of three polypeptide chains. *J. Immunol.* In press.
36. Kitamura, H., M. Matsumoto, and S. Inai. 1976. Necessity of the divalent cation in the reaction between EAC1-8hu and C9gp. *Int. Arch. Allergy Appl. Immunol.* **51:** 265–271.