Absence of the Eighth Component of Complement in Association with Systemic Lupus Erythematosus-Like Disease

HUGO E. JASIN

From the Rheumatic Diseases Group, Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas, Texas 75235

ABSTRACT A 56-yr-old black woman with absence of the eighth component of complement and a disease compatible with systemic lupus erythematosus is described. Her disease is characterized by the presence of photosensitive "malar" rash, alopecia, polyarthritis, and nephrotic syndrome.

Hemolytic and immunochemical assays of the serum complement components were normal, except for C8 which was undetectable. Hemolytic activity could be restored to normal by the addition of functionally pure C8. In vitro tests to investigate the functional integrity of the classical and alternative pathways indicated that the functions mediated by activation of C3 and C5 were intact whereas heat-labile bactericidal activity was totally absent. Addition of C8 restored this function to normal levels.

One of two brothers of the proband was shown to have serum C8 levels approaching 50% of normal indicating the hereditary nature of the defect. HLA typing studies showed that the normal brother had identical haplotypes to the proband while the proposed heterozygous brother only shared one haplotype with the patient, suggesting that the gene controlling the C8 defect was not closely linked to the major histocompatibility complex. If the association of a connective tissue disease and absence of a terminal component of complement is not coincidental, these results suggest that C8 deficiency may be associated with a subtle defect in the defense mechanisms to viral infection leading to viral persistance and perhaps to diseases such as systemic lupus erythematosus where chronic viral infections have been implicated.

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INTRODUCTION

Hereditary deficiencies of most of the individual complement components of the hemolytic sequence have been described in man (1, 2). Of interest is the unexpected detection of such complement deficiencies in patients with connective tissue diseases, particularly systemic lupus erythematosus (SLE).[1] This association has been found most commonly in patients with homozygous and heterozygous C2 deficiencies (1, 3) and in two patients with defects of the terminal membrane attack complex (C5–9) (4, 5).[2]

Hereditary deficiency of the eighth component of complement has been previously described in one patient with disseminated gonococcemia (6, 7). We report here a second patient with C8 deficiency and associated SLE, the immunochemical and functional characteristics of the patient's serum, and the probable mode of inheritance of the defect. This unusual association in a patient where most complement-mediated functions except membrane lysis were found to be intact provides some insight on the possible mechanisms which may play a role in the pathogenesis of SLE.

PATIENT HISTORY

I. C. is a 56-yr-old black woman admitted to Parkland Memorial Hospital in November 1971 for evaluation of her renal disease. She had first been seen in April 1956 complaining of pleuritic chest pain, and soon afterwards she developed nephrotic syndrome with a 24-h urinary protein of 5.9 g. At that time LE prepara-

[1] *Abbreviations used in this paper*: BSA, bovine serum albumin; CH_{50}, hemolytic complement; MHC, major histocompatibility complex; SLE, systemic lupus erythematosus.

[2] Nomenclature used for the classical complement pathway conforms to that agreed upon by the World Health Organization in 1968. (*Bull. W. H. O.* **39:** 935–938.).

tions were negative. In December 1957 she was admitted to the hospital with a history of weight loss of 10 pounds, alopecia, polyarthritis, fever, and a photosensitive facial rash. She had frontal alopecia, a maculopapular rash on face and forehead, generalized lymphoadenopathy, and a sterile pleural effusion. Laboratory findings: hemoglobin, 9.6 g/100 ml; normal blood leukocyte count; blood urea nitrogen, 27 mg/100 ml; serum creatinine, 2.2 mg/100 ml. LE preparations were again negative. The patient was given 15 mg Prednisone daily with rapid control of the fever. In 1958 the photosensitive facial rash reappeared 2 mo after discontinuation of corticosteroid therapy. In April 1959 LE preparations were positive on three consecutive determinations, and the sensitized sheep erythrocyte agglutination test was also positive at a titer of 1/56. In 1960 she continued to have proteinuria of 4.08 g/24 h with a creatinine clearance of 49 ml/min. Red cell sedimentation rate (Westergren) was 129 mm/h. Renal biopsy showed diffuse capillary basal membrane thickening (wire loops) and arteriolar nephrosclerosis. From 1962 to 1968 the patient was practically asymptomatic. Prednisone was slowly decreased and finally discontinued in 1965. Family history was noncontributory; the three surviving brothers and a sister are in apparent good health.

In November 1971 she was admitted for evaluation of her renal status. No significant physical findings were recorded. Laboratory findings: blood urea nitrogen, 46–55 mg/100 ml; serum creatine, 2.0 mg/100 ml; creatinine clearance, 48 ml/min; 24 h proteinuria, 3.2 and 2.1 g/24 h. LE preparations were negative; in fact, after the positive results obtained in 1959, subsequent tests up to the present have yielded negative results. Fluorescent antinuclear antibody tests have also been repeatedly negative since first tested in 1961. Slide latex fixation test was positive, and sensitized sheep cell agglutination was positive 1/28. Hemolytic complement determination (CH50), <5U (normal 60–110 U); serum C3, 140–170 mg/100 ml (normal 100–180 mg/100 ml). Renal biopsy showed a picture compatible with advanced chronic glomerulonephritis by light microscopy. Immunofluorescence examination of frozen sections showed focal deposits of IgG, IgA, and IgM on the glomerular basal membrane, and staining for fibrin was negative. Immunofluorescence studies done on normal skin failed to detect immunoglobulin or complement deposits under the basal membrane. In 1972 the patient was given 20 mg Prednisone and 100 mg cyclophosphamide daily. In spite of this therapeutic regime, her renal function progressively deteriorated over a 2-yr period, and she developed end-stage renal disease. At the present time she undergoes hemodialysis three times weekly.

METHODS

Sera. Human sera were obtained from blood drawn by venipuncture that was allowed to clot for 1 h at room temperature, separated, and stored at −70°C.

Complement components. Functionally pure complement components were obtained from Cordis Laboratories, Miami, Fla. In addition, a crude preparation of human C1 was prepared according to the method of Nelson et al. (8). C5 was prepared according to the methods of Nilsson and Müller Eberhard (9). Partially purified antiserum to human C8 was obtained from Atlantic Antibodies, Westbrook, Maine. This antiserum could be made monospecific for C8 by the addition of 1:5 vol:vol of C8-deficient serum. Monospecific antihuman C3 serum was obtained from Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif.

Complement assays. Total hemolytic complement was measured by microtitration according to the technique of Nelson et al. (8). Hemolytic titrations of C1 and C3–C9 were performed according to the method of Nelson et al. (8) with reagents provided by Cordis Laboratories. Hemolytic C4 and C2 titrations were done with C4-deficient guinea pig serum (10) and C2-deficient human serum (11). For every assay at least five normal sera were used to determine the normal range. The serum complement component levels were also measured immunochemically by the single radial immunodiffusion technique (12). C1S, C4, and C5–C9 measurements were performed by Dr. Hans Müller Eberhard. C4 and C3 plates were obtained from Hyland Div., Travenol Laboratories, Inc., and Factor B plates were from Behring Diagnostics, American Hoechst Corp., Somerville, N. J. C3, C2, and Factor B allotyping was performed by Dr. Chester Alper, Center for Blood Research, Boston, Mass.

Complement-dependent functions. Serum heat-labile opsonic capacity using *Escherichia coli* 075 was assayed as previously described (13, 14). The functional integrity of the alternative pathway of complement activation was also assayed by incubation of serum with inulin at a concentration of 10 mg/ml at 37°C for 45 min (15). The treated serum and a control containing 0.01 M EDTA were analyzed for the presence of C3 fragments by crossed immunoelectrophoresis (16).

Serum bactericidal capacity was assayed by a turbidimetric method with *E. coli* 0111B4 according to the method of Rother et al. (17).

Serum chemotactic activity was generated by incubation of fresh or heat-inactivated sera with 0.1 mg/ml of a bovine serum albumin (BSA), rabbit anti-BSA antigen-antibody precipitate formed at immune equivalence. The sera were incubated at 37°C for 45 min, and residual complement was then inactivated by heating at 56°C for an additional 30 min (18). Chemotaxis was measured by a modified Boyden technique (18, 19) with disposable polystyrene chambers (Adaps Inc., Dedham, Mass.) and micropore filters 3-μm pore size (Millipore Corp., Bedford, Mass.).

Solubilization of immune precipitates was performed according to the method of Czop and Nussenzweig (20) with immune precipitates containing ^{125}I-BSA labeled by the method of MacFarlane (21). Results were expressed as percent of solubilization (100 × cpm in supernatant fluids/total cpm).

Immunologic studies. Tissue typing was performed by Dr. Peter Stastny with a standard microcytotoxicity test (22). Double immunodiffusion was performed with 0.6% agarose gels in cacodylate buffer, pH 7.2 (23).

RESULTS

Characterization of the C8 deficiency. Repeated determinations of total hemolytic complement since 1972 had shown undetectable hemolytic activity in the patient's serum and plasma. When the individual complement components were assayed functionally and immunochemically it was apparent that all components except C8 were within the normal range. C8 hemolytic activity was less than 0.3% of the lower limit of the normal range, and no precipitin rings were formed when the serum was assayed by radial immunodiffusion (Table I). The sera used for these measurements were obtained when the patient was clinically inactive and the normal levels of the early complement components provide confirmation for this clinical impression.

Reconstitution of the normal hemolytic activity was attempted by the addition of several functionally pure complement components. Addition of C1, C4, C2, C3, C5, C6, and C7 and C9 fail to reconstitute hemolytic activity, while addition of 50 U of functionally pure C8 restored total hemolytic activity to the normal level of 80 U. Addition of equal volumes of C8-deficient serum from a different patient (6) also failed to restore activity.

Further confirmation of the integrity of the rest of the hemolytic sequence in the patient's serum was obtained by the preparation of EAC1-7 cells. EA were incubated at 37°C for 45 min in the presence of C8-deficient serum diluted to 1/200. The erythrocytes were washed and assayed. As seen in Table II, hemolytic activity was only seen if both terminal components C8 and C9 were added or if the serum was reconstituted by the addition of C8.

Attempts to detect C8 inhibitory activity in the patient's serum were unsuccessful. Hemolytic titration of purified C8 was carried in the presence of C8 deficient serum at a final dilution of 1:50. The stoichiometric titration yielded a linear plot suggesting absence of a C8 inhibitor. Furthermore, titration after incubation of a mixture of equal volumes of normal serum and C8-deficient serum showed no detectable diminution of hemolytic activity.

Complement-mediated functions. Several complement mediated functions were tested in vitro to investigate the functional integrity of the activating sequence and of the functions mediated by the third and fifth components of complement. Chemotactic activity was generated by incubating sera with preformed immune complexes. The results obtained indicated that the chemotactic activity generated by serum I.C. was identical to the activity of normal serum.

Heat-labile opsonic capacity was also tested with *E. coli* 075 as the microorganism. This test investigates both the integrity of the alternative pathway and opsonization mediated by C3b (13, 24). The results obtained indicated that this function was perfectly normal in serum I.C. at 5% concentration suggesting that activation of the alternative pathway as well as C3 were functionally normal. These results were confirmed by the use of inulin to activate the alternative pathway. Analysis of the resulting C3 products by crossed immunoelectrophoresis showed no difference

TABLE I
Individual Complement Components of the C8-Deficient Patient and Two Brothers

	Hemolytic assay				Immunodiffusion			
Component	I. C.	E. G.	R. G.	Normal range	I. C.	E. G.	R. G.	Normal range
	Units				*μg/ml*			
CH50	<5	30	70	60–110	—	—	—	—
C1	60,000	60,000	30,000	15,000–60,000	—	—	—	—
C1q	—	—	—	—	154	138	154	136–169
C1s	—	—	—	—	89	92	120	64–87
C4	153,000	150,000	230,000	76,000–614,000	418	430	426	456–745
C2	3,050	1,600	4,800	1,000–4,000	ND*	ND	ND	—
C3	24,000	ND	ND	16,000–64,000	1,400	1,225	1,960	1,050–1,800
C5	120,000	80,000	ND	60,000–180,000	118	100	190	68–83
C6	120,000	120,000	ND	80,000–320,000	87	49	92	63–78
C7	120,000	120,000	ND	60,000–240,000	113	74	87	45–64
C8	<500	85,000	290,000	150,000–300,000	NR‡	36	74	46–62
C9	60,000	240,000	480,000	40,000–240,000	270	195	350	160–260
Factor B	—	—	—	—	176	230	270	130–250

* ND, not done.

‡ NR, precipitin rings not detectable.

TABLE II
*Hemolytic Assay Using EAC1–7 Cells**

Component added	Hemolytic units
C8‡	<50
C9‡	<50
C8+C9‡	1,200
Serum I. C.	<50
Serum I. C.+C8§	9,600

* EA were incubated with a 1/200 dilution of C8-deficient serum.
‡ 500 U of C8 or C9 added.
§ 500 U per well added.

between normal sera and the C8-deficient serum. The degree of complement-dependent solubilization of immune complexes (25) obtained with C8-deficient serum was identical to that of normal sera.

Serum bactericidal capacity was tested because it had been previously shown to depend upon the integrity of the whole sequence of complement activation including the membrane attack complex C5–C9 (26). *E. coli* 0111B4 was lysed by most normal sera in the absence of specific antibody. However, I.C. serum was not only unable to kill the bacteria, but bacteria actually grew during the hour of incubation at 37°C. Addition to the patient's serum of 100 U/ml of functionally purified C8 restored bactericidal capacity to normal. Thus, only hemolytic and bactericidal capacities were found to be deficient in the patient's serum.

Family studies. Of the patient's four living relatives, only two brothers were available for study. As shown in Table I, one brother (R.G.) had normal levels of total hemolytic complement and individual components including C8, while the other (E.G.) showed low hemolytic complement and roughly one-half of the normal concentration of C8 both by hemolytic titration and immunodiffusion. Hemolytic C8 titrations of serum from the proband and the two brothers were performed. E.G., the proposed heterozygote, exhibited stoichiometric C8 titrations with a calculated C8 hemolytic activity of about 50% of normal, whereas I.C. showed no detectable activity. Double immunodiffusion analysis with a monospecific C8 antiserum (Fig. 1) showed decreased amounts of immunoreactive C8 in E.G. and normal levels in the other brother's serum. The proband's serum not only failed to show any reactivity, but no soluble interfering material was detected as judged by inspection of the adjacent precipitin arcs.

Possible linkage of the gene controlling C8 deficiency and the major histocompatibility region was investigated. HLA typing studies (Fig. 2) showed that the brother with normal hemolytic and immunochemical levels of C8 (R.G.) had identical haplotypes to the homozygous proband whereas E.G., the proposed

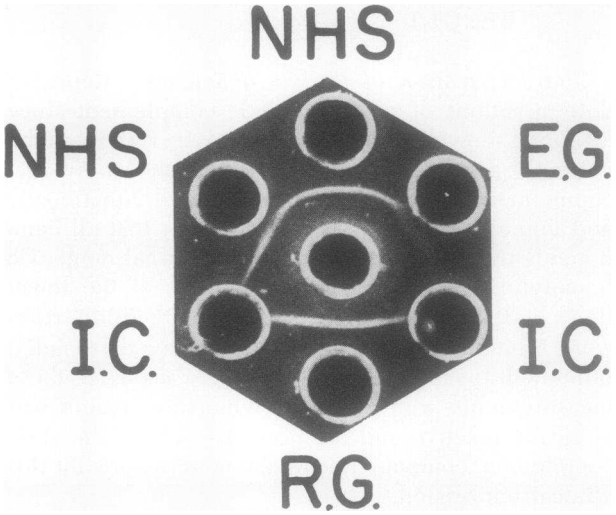
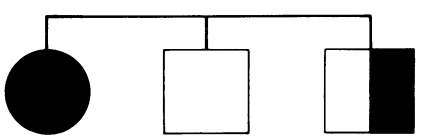


FIGURE 1 Double-diffusion analysis in agarose gel. The center well contains monospecific anti-C8 serum. E.G. is the proposed heterozygote and R.G. is the normal brother. NHS = normal human serum. No precipitin line formed with the proband's serum (I.C.), whereas a weak line is seen with serum E.G.

heterozygous brother, only shared one haplotype with the patient. Genetic analysis of C2 and Factor B allotypes was not informative since the three members of the family showed identical allotypes.

DISCUSSION

The patient described in this report demonstrates the association of complete absence of the eighth component of complement and a disease complex compatible with the diagnosis of SLE according to established criteria (27). Serum levels of the individual



	I.C.	R.G.	E.G.
C8 (H)	<500U	290,000U	85,000U
C8 (I)	0 mg/100 mg	74 mg/100 mg	36 mg/100 mg
Haplotypes	AW19 B12 AW29 BW35	AW19 B12 AW29 BW35	AW19 B12 A28 BW22

FIGURE 2 Genetic studies on C8-deficient family. The brother with normal hemolytic (H) and immunochemical (I) levels of C8 (R.G.) shows identical haplotypes to the homozygous proband while the proposed heterozygous brother (E.G.) with reduced C8 levels shares only one haplotype with the proband.

complement components except for C8 were normal both by functional and immunochemical methods. That the activating sequence of hemolytic complement was intact, at least up to the seventh component, was shown by the capacity of the C8-deficient serum to generate sensitized sheep erythrocytes lysable only by the simultaneous addition of functionally pure C8 and C9 (EAC1-7). Furthermore, the functional tests requiring the integrity of the alternative pathway, C3 and C5 such as heat-labile opsonic capacity, solubilization of immune complexes (20), C3 breakdown upon incubation with inulin (15), and generation of chemotactic factors (28, 29) were also shown to be normal in the patient's serum. Thus, only functions such as hemolysis and bacterial lysis, which are dependent upon the generation of functionally active membrane attack complex C5–9 (26, 30), were found to be absent in the patient's serum. Inherited deficiencies of the individual components of complement except for C9 have been described (1, 2) and one patient with isolated C8 deficiency and disseminated gonococcal infection has been previously reported (6). The immunochemical and functional characteristics of the sera from both patients seem to be quite similar, and, furthermore, combination of both sera failed to reconstitute hemolytic activity suggesting that the defect in both was of identical nature.

Our patient has a 20-yr history of a disease characterized by the presence of membranous glomerulonephritis and clinical features compatible with SLE, but she showed positive serologic tests (LE preparations) only during one admission early in the disease. Atypical or absent serologic features in patients with SLE and complement component deficiencies have been noticed previously (3, 31).

In spite of the limited number of family members available for study, the results obtained with HLA typing were informative. The existence of a brother with one-half of the normal levels of serum C8 indicates that the defect under study has a genetic basis and that, as has been shown previously for the other complement deficiencies (1, 2), it may be transmitted as an autosomal codominant defect. The HLA typing studies are more difficult to interpret. The presence of identical haplotypes in the proband and brother with normal C8 levels strongly suggest that the gene controlling the C8 defect may not be closely linked to the major histocompatibility complex (MHC) since the alternative conclusion would assume separate cross-over events taking place in each member of the chromosome pair. However, a higher rate of cross-over events in that region of the genome may occur in family members affected with SLE (32) so that, even though the available information is strongly suggestive for the absence of close linkage between the abnormal C8 gene and the MHC, the unavailability of the proband's progenitors or of a more extensive pedigree does not permit a definitive conclusion on this issue. Merritt et al. (7) have shown close linkage of C8 with the MHC in an analysis of the pedigree of another patient with C8 deficiency (6). Another family with the same defect has been under study and in this latter case, the C8 gene was not linked to the MHC (33). Recent evidence has shown that C8 is composed of three polypeptide chains of different molecular weights (34). Thus, the complex molecular structure of C8 precludes the designation of a unique specific locus for the C8 deficiency, and C8-deficient phenotypes may result from different genetic defects localized in different regions of the genome.

The numerous reports describing association of immune complex or connective tissue diseases with hereditary complement component deficiencies (1, 2) have stimulated much recent interest. The occurrence of such hereditary defects in the general population is rare (3) so that in spite of the very serious problems of ascertainment generated by selection bias in the case of patients affected with rheumatic disease, most investigators have accepted the hypothesis that this association is not accidental (3). Rheumatic diseases have been mostly associated with deficiency of the early acting components, particularly C2 (1–3). In patients with deficiencies of the late-acting components, association with connective tissue diseases has only been rarely reported (4, 5). Many hypotheses have been proposed to explain this association (35–41), but so far there is no direct evidence to support any of them. Recently, the complement system has been shown to be involved in viral neutralization (42–44), and complement defects may therefore contribute to viral persistence in patients with SLE. If the association of C8 deficiency and SLE seen in our patient is not coincidental, our findings would support this mechanism as a possible pathogenic factor since it is likely that the complement-dependent functions such as handling of immune complexes, defense mechanisms against most bacterial infections, and immune responses may be unaffected in this patient. Furthermore, our genetic studies suggest that the gene controlling C8 deficiency may not be closely linked to the MHC in this patient. These findings suggest that patients such as the one described here may have a subtle defect in their defense mechanisms leading to persistence of virus and of virus infected cells and perhaps to diseases such as SLE where chronic viral infections have been implicated (45).

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REFERENCES

1. Stroud, R. M. 1974. Genetic abnormalities of the complement system of man associated with disease. *Transplant. Proc.* **6:** 59–65.
2. Polley, M. J., and A. G. Bearn. 1975. Genetic aspects of diseases of complement: an explosion. *Am. J. Med.* **58:** 105–111.
3. Glass, D., D. Raum, D. Gibson, J. S. Stillman, and P. H. Schur. 1976. Inherited deficiency of the second component of complement. Rheumatic disease associations. *J. Clin. Invest.* **58:** 853–861.
4. Rosenfeld, S. I., M. E. Kelly, and J. P. Leddy. 1976. Hereditary deficiency of the fifth component of complement in man. I. Clinical, immunochemical, and family studies. *J. Clin. Invest.* **57:** 1626–1634.
5. Boyer, J. T., E. P. Gall, M. E. Norman, U. R. Nilsson, and T. M. Zimmerman. 1975. Hereditary deficiency of the seventh component of complement. *J. Clin. Invest.* **56:** 905–913.
6. 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.
7. Merritt, A. D., B. H. Petersen, A. A. Biegel, D. A. Mayers, G. F. Brooks, and M. E. Hodes. 1976. Chromosome 6: linkage of the eighth component of complement (C8) to the histocompatibility region (HLA). *Cytogenet. Cell Genet.* **16:** 331–334.
8. 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.
9. Nilsson, U. R., and H. J. Müller Eberhard. 1965. Isolation of Bl_F globulin from human serum and its characterization as the fifth component of complement. *J. Exp. Med.* **122:** 277–298.
10. Gaither, T. A., D. A. Alling, and M. M. Frank. 1974. A new one-step method for the functional assay of the fourth component (C4) of human and guinea pig complement. *J. Immunol.* **113:** 574–583.
11. Ngan, B. Y., E. W. Gelfand, and J. O. Minta. 1976. A one-step method for molecular quantitation of C2. *Fed. Proc.* **35:** 439. (Abstr.)
12. Mancini, G., A. O. Carbonara, and J. F. Heremans. 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry.* **2:** 235–254.
13. Jasin, H. E. 1972. Human heat labile opsonins: evidence for their mediation via the alternative pathway of complement activation. *J. Immunol.* **109:** 26–31.
14. Jasin, H. E., J. H. Orozco, and M. Ziff. 1974. Serum heat labile opsonins in systemic lupus erythematosus. *J. Clin. Invest.* **53:** 343–353.
15. Götze, O., and H. J. Müller-Eberhard. 1971. The C3-activator system: An alternative pathway of complement activation. *J. Exp. Med.* **134:** 90s–108s.
16. Laurell, C. B. 1965. Antigen-antibody crossed electrophoresis. *Anal. Biochem.* **10:** 358–361.
17. Rother, K., U. Rother, K. F. Peterson, D. Gemsa, and F. Mitze. 1964. Immune bactericidal activity of complement. Separation and description of intermediate steps. *J. Immunol.* **93:** 319–330.
18. Clark, R. A., M. M. Frank, and H. R. Kimball. 1973. Generation of chemotactic factors in guinea pig serum via activation of the classical and alternate complement pathways. *Clin. Immunol. Immunopathol.* **1:** 414–426.
19. Boyden, S. 1962. The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leukocytes. *J. Exp. Med.* **115:** 453–466.
20. Czop, J., and V. Nussenzweig. 1976. Studies on the mechanism of solubilization of immune precipitates by serum. *J. Exp. Med.* **143:** 615–630.
21. MacFarlane, A. S. 1963. In vivo behavior of I^{131} fibrinogen. *J. Clin. Invest.* **42:** 346–361.
22. Mittal, K. K., M. R. Mickey, D. P. Singal, and P. I. Terasaki. 1968. Serotyping for homotransplantation. XVIII. Refinement of microdroplet lymphocyte cytotoxicity test. *Transplantation (Baltimore).* **6:** 913–927.
23. Agnello, V., R. J. Winchester, and H. G. Kunkel. 1970. Precipitin reactions on the Clq component of complement with aggregated γ globulin and immune complexes in gel diffusion. *Immunology.* **19:** 909–919.
24. Gigli, I., and R. A. Nelson, Jr. 1968. Complement dependent-immune phagocytosis. I. Requirement for C'1, C'4, C'2, C'3. *Exp. Cell Res.* **51:** 45–67.
25. Miller, G. W., and V. Nussenzweig. 1975. A new complement function: solubilization of antigen-antibody aggregates. *Proc. Natl. Acad. Sci. U. S. A.* **72:** 418–422.
26. Goldman, J. N., S. Ruddy, K. F. Austen, and D. S. Feingold. 1969. The serum bactericidal reaction. IV. Antibody and complement requirement for killing a rough *Escherichia coli*. *J. Immunol.* **102:** 1379–1387.
27. Cohen, A. S., W. E. Reynolds, E. C. Franklin, J. P. Kulka, M. W. Ropes, L. E. Shulman, and S. L. Wallace. 1971. Preliminary criteria for the classification of systemic lupus erythematosus. *Bull. Rheum. Dis.* **21:** 643–648.
28. Ward, P. A., C. G. Cochrane, and H. J. Müller-Eberhard. 1965. The role of serum complement in chemotaxis of leukocytes in vitro. *J. Exp. Med.* **122:** 327–346.
29. Snyderman, R., J. K. Phillips, and S. E. Mergenhagen. 1971. Biological activity of complement in vivo. Role of C5 in the accumulation of polymorphonuclear leukocytes in inflammatory exudates. *J. Exp. Med.* **134:** 1131–1143.
30. Stolfi, R. L. 1968. Immune transformation: a state of irreversible damage generated as a result of the reaction of the eight components in the guinea pig complement system. *J. Immunol.* **100:** 46–54.
31. Hauptmann, G., E. Grosshans, and E. Reid. 1974. Lupus erithemateux aigus et déficits hereditaires en complement. A propos d'un cas par déficit complet en C4. *Ann. Dermatol. Syphiligr.* **101:** 479–496.
32. Raum, D., D. Glass, C. B. Carpenter, C. A. Alper, and P. H. Schur. 1976. The chromosomal order of genes controlling the major histocompatibility complex, Properdin, factor B, and deficiency of the second component of complement. *J. Clin. Invest.* **58:** 1240–1248.
33. Giraldo, G., L. Degos, E. Beth, N. Sasportes, A. Marcelli, R. Garbi, and N. K. Day. 1977. C8 deficiency in a family with xeroderma pigmentosum. Lack of linkage to the HLA region. *Clin. Immunol. Immunopathol.* In press.
34. Kolb, W. P., and H. J. Müller-Eberhard. 1976. The membrane attack mechanism of complement: the three polypeptide chain structure of the eighth component (C8). *J. Exp. Med.* **143:** 1131–1140.
35. Kohler, P. F. 1975. Inherited deficiencies and systemic

lupus erythematosus: an immunogenetic puzzle. *Ann. Intern. Med.* **82:** 420–421.

36. Démant, P., J. Capková, E. Hinzová, and B. Vorácová. 1973. The role of the histocompatibility-2-linked Ss-Slp region in the control of mouse complement. *Proc. Natl. Acad. Sci. U. S. A.* **70:** 863–864.
37. Ferreira, A., and V. Nussenzweig. 1975. Genetic linkage between serum levels of the third component of complement and the H-2 complex. *J. Exp. Med.* **141:** 513–517.
38. Fu, S. M., H. G. Kunkel, H. P. Brusman, F. H. Allen, Jr., and M. Fotino. 1975. Evidence for linkage between HLA histocompatibility genes and those involved in the synthesis of the second component of complement. *J. Exp. Med.* **140:** 1108–1111.
39. Day, N. K., P. L'Esperance, R. A. Good, A. F. Michael, J. A. Hansen, B. Dupont, and C. Jersild. 1975. Hereditary C2 deficiency: genetic studies and association with the HL-A system. *J. Exp. Med.* **141:** 1464–1469.
40. Allen, F. H., Jr. 1974. Linkage of HL-A and GBG. *Vox Sang.* **27:** 382–384.
41. Lilly, F. 1971. The influence of H-2 types on Gross virus leukemogenesis in mice. *Transplant. Proc.* **3:** 1239–1241.
42. Daniels, C. A., T. Borsos, H. J. Rapp, R. Snyderman, and A. L. Notkins. 1970. Neutralization of sensitized virus by purified components of complement. *Proc. Natl. Acad. Sci. U. S. A.* **65:** 528–535.
43. Cooper, N. R., F. C. Jensen, R. M. Welsh, and M. B. A. Oldstone. 1976. Lysis of RNA tumor viruses by human serum: direct antibody-independent triggering of the classical complement pathway. *J. Exp. Med.* **144:** 970–984.
44. Theofilopoulos, A. N., and L. H. Perrin. 1976. Lysis of human lymphoblastoid cells by self-activation of the alternative complement pathway. *Fed. Proc.* **35:** 493. (Abstr.)
45. Christian, C. L. 1976. Systemic lupus erythematosus and type C-RNA viruses. *N. Engl. J. Med.* **295:** 501–502.