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Neisseria meningitidis Bacteremia in Association with Deficiency of the Sixth Component of Complement

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The serum of a 26-year-old black man with a recent episode of meningococcemia complicated by meningitis and arthritis was found to lack hemolytic complement activity. The sixth component of complement was not detected by functional or immunochemical assays whereas other components were normal by hemolytic assay. His fresh acute-phase serum lacked complement-mediated bactericidal activity against the homologous strain of *Neisseria meningitidis*, but the addition of fresh normal serum or purified C6 restored bactericidal activity as well as hemolytic activity. The absence of C6 activity could not be accounted for on the basis of an inhibitor. Opsonization and chemotaxis functioned normally. Histocompatibility typing of family members did not demonstrate evidence for genetic linkage of C6 deficiency with the major histocompatibility loci. This report represents the first published case of C6 deficiency associated with bacteremic *Neisseria* infections in which antimeningococcal bactericidal antibodies have been definitively demonstrated against the homologous strain in the acute phase of the illness.

Several investigators have recently observed that patients with deficiencies of the terminal components of the complement cascade (C5, C6, C7, and C8) have a predisposition for bacteremic *Neisseria* infections (8-10, 15, 17; B. H. Petersen, T. J. Lee, R. Snyderman, and G. F. Brooks, Ann. Intern. Med., in press). Except for the C5 deficiency, these individuals possess normal complement-mediated opsonization and chemotaxis but lack bactericidal activity (8-10, 15). This report described a hereditary deficiency of the sixth component of complement in a man who presented with meningococcal bacteremia, meningitis, and arthritis.

MATERIALS AND METHODS

Patients. Patient C.A., a 26-year-old black man, was admitted to North Carolina Memorial Hospital April 1977 on transfer from his local hospital with *Neisseria meningitidis* bacteremia, meningitis, and arthritis. There was no previous history of meningitis or other serious infectious complications. Three other siblings (8 through 26 years of age) are alive and well. There was no history of consanguinity. Both of his parents have been free of unusual infectious diseases.

Pertinent laboratory data included immunoglobulin (Ig) determinations; IgG was elevated at 2,030 mg/100 ml (normal range, 535-1620). The IgA and IgM levels were normal: 225 mg/100 ml (normal range, 75-370) and 146 mg/100 ml (normal range, 25-210), respectively. The prothrombin time was 12.6 s with a control of 12.5 s; the partial thromboplastin time was 48.8 s with a control of 55.4 s; and total clotting time was 11.2 s with a control of 11.6 s. The platelet count was 204,500/mm^3, and the Ivey bleeding time was 4 min. After institution of aqueous penicillin, 20×10^6 U administered intravenously in divided doses per day to complete a total course of 14 days, the patient had an uneventful recovery with no subsequent complications, relapses, or sequelae.

Complement assays. Human sera used for the complement assays were either freshly drawn or stored frozen in 0.5-ml aliquots at −70°C. Complement donor sera used in the bactericidal assays were obtained from an 18-year-old hypogammaglobulinemic patient who had a normal level of total hemolytic complement (CH_{50}) or 40 U/ml and no bactericidal activity against the homologous strain.

Monospecific rabbit antiserum to human C6 was obtained from Hyland Laboratories (Costa Mesa, Calif.) prepared as previously described (1). Monospecific antisera to other complement components were provided by Hans J. Müller-Eberhard and Carlos Arroyave, Scripps Clinic and Research Foundation, La Jolla, Calif., or were obtained from Hyland Laboratories.

Titration of CH_{50} was performed by the method of Mayer (12), and functional assays of the complement components (C1 through C9) were performed with modifications of the methods of Nelson et al. (14) as

previously described (9). All titrations of C6 were done in the test tube assay, and other complement components were measured in the microtiter assay. Immunochemical quantification of individual components in 19 normal controls and a normal serum pool was performed by single radial diffusion (1, 11) as previously described (9). Functionally pure C6 was obtained from Cordis Corporation.

Complement-dependent bactericidal activity was assayed as previously described (9) utilizing *N. meningitidis* (group Y) cultured from the patient's blood. In vitro phagocytosis of the homologous strain and *Staphylococcus aureus* 502A by human peripheral white blood cells was analyzed by a modification of the technique of Pincus and Klebanoff (16) as recently described (9). Polymorphonuclear leukocyte suspensions from a normal donor were prepared as described by Böyum (2). The generation of chemotactic activity in serum was quantified, using methods previously described, in modified Boyden chambers (18). Histocompatibility (HLA) typing was performed by Fran Ward by the microcytotoxicity assay.

RESULTS

In analysis of host factors that predispose to bacteremic neisserial infections, this proband represented the second patient of 30 studied whose serum was completely deficient in CH_{50} hemolytic activity (Table 1). The first patient was previously reported and was found to have C7 deficiency (9). Further analysis revealed no C6 hemolytic activity in the serum; all other complement components measured were within the normal range. No immunoreactive C6 was detected by a single radial immunodiffusion with monospecific rabbit antiserum to C6 (Table 1). Normal serum used as a reference contained ~120 μg of C6 per ml. Thus, the results showed that the patient was totally deficient in functional and immunoreactive C6. The addition of functionally pure C6 (2,000 U/ml) to the patient's serum restored CH_{50} activity from undetectable to normal levels; the CH_{50} activity of normal serum was not altered after incubation for 60 min with an equal volume of the patient's undiluted serum.

Bactericidal activity against the homologous strain of *N. meningitidis* was completely lacking. The addition of hypogammaglobulinemic serum with normal CH_{50} activity or functionally pure C6 to acute-phase serum from the patient restored bactericidal activity, and bactericidal titers of 1:16 against this strain were noted. The heat-inactivated C6-deficient serum alone, the C6-deficient serum alone, the complement donor serum alone, and functionally pure C6 alone did not exhibit bactericidal activity (Fig. 1).

Opsonization, phagocytosis, and killing of *S. aureus* 502A and the homologous strain were normal, and results were similar to those obtained with the reference serum. The C6-deficient serum supported the generation of normal amounts of chemotactic activity when activated by either heat-aggregated human gamma globulin or zymosan (Table 2). These data indicate that deficiency of C6 does not affect opsonization and does not depress the production of chemotactic activity upon activation of the complement system by either the classical or alternative pathways.

The CH_{50} and C6 functional titers and the immunochemical determination of C6 levels of the family members are listed in Table 1. Ap-

TABLE 1. *Whole complement and C6 levels of the C6-deficient patient and family members*

Family member	Whole complement (U/ml)	C6 hemolytic assay (U/ml)	C6 immunochemical assay (mg/ml)
I-1	45	52,000	0.093
I-2	47	72,000	0.135
II-1	39	59,000	0.126
II-4	0	<10	<0.03
Normal controls	24-45[a]	100,000-120,000[b]	0.120[c]

[a] 29 normal controls, 35 ± 10 (2 standard deviations [SD]).
[b] 22 normal controls, 110,000 ± 10,000 (2 SD).
[c] 19 normal controls, 0.12 ± 0.03 (2 SD).

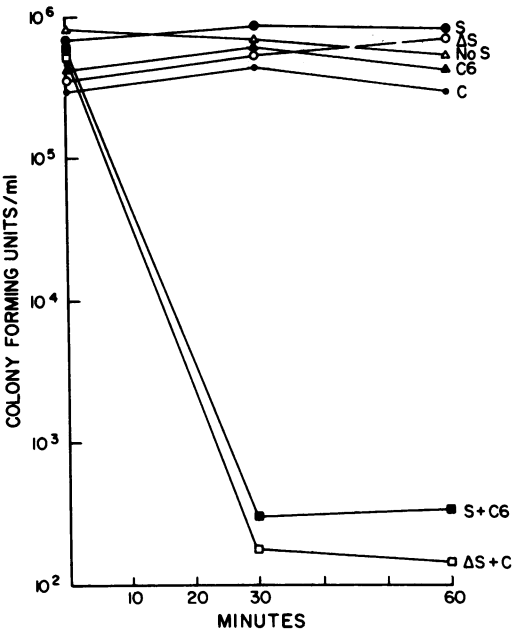

FIG. 1. *Bactericidal activity of C6-deficient (C6d) serum against the homologous strain (Neisseria meningitidis group Y). C6d serum, buffer (noS), complement donor serum (C), and purified C6 alone did not exhibit bactericidal activity. The addition of (C) containing 25 CH_{50} units of total hemolytic complement or purified C6 to C6d serum reconstituted bactericidal activity.*

TABLE 2. *Generation of chemotactic activity in C6-deficient serum*[a]

Expt	Individual tested	Chemotactic activity produced[b]			
		10 min		60 min	
		AHGG	Zymosan	AHGG	Zymosan
1	Normal	72.3	24.4	64.7	21.5
	C6-deficient patient	63.0	84.5	106.9	60.6
2	Normal 1	—[c]	—	30.7	74.6
	Normal 2	—	—	27.1	46.9
	Normal 3	—	—	39.3	57.4
	C6-deficient patient	—	—	29.7	64.7

[a] Serum from the indicated individuals was diluted 1 to 2.5 in gelatin-Veronal buffer (GVB^{++}) and then incubated at 37°C for the indicated time periods with either 1 mg of aggregated human gamma globulin (AHGG), 1 mg of zymosan, or buffer alone. After incubation, the sera were heated at 56°C for 30 min, centrifuged (2,000 × *g* for 15 min) to remove particulate material, and then tested for chemotactic activity. The appropriate background chemotactic activity of serum plus buffer alone was subtracted from the values shown.

[b] The mean of triplicate samples expressed as migrated polymorphonuclear leukocytes per microscopic field (×640).

[c] —, Not done.

proximately half-normal serum levels of functional C6 were noted in the proband's mother (I-1), father (I-2), and sister (II-1) respectively. These individuals have thus been designated as probable heterozygotes for C6 deficiency on the basis of 50% levels by hemolytic analysis although immunochemical determinations were within the normal range (Table 1). The HLA typing for each family member is depicted in the pedigree (Fig. 2). The proband, an obligate homozygote for C6 deficiency, was heterozygous for the Aw26-B39 and Aw33-Bw35-Cw4 haplotypes. Close linkage of HLA loci and C6 deficiency would thus require linkage to two different haplotypes, Aw26-B39 and Aw33-Bw35-Cw4. A sibling of identical HLA type had 50% levels of C6 hemolytic activity and is presumably heterozygous for C6 deficiency, as are the parents. Therefore, no support was evident for linkage of the locus for C6 deficiency to the HLA loci, although some family members were not available for study.

DISCUSSION

There have been five previous reports of C6 deficiency in humans associated with bacteremic *Neisseria* infections including two published cases (8, 10; Petersen et al., in press), but this report presents only the second case in which antimeningococcal bactericidal activity was restored in the acute-phase serum. Gold et al. described a 6-year-old boy with C6 deficiency and chronic meningococcemia and restoration of bactericidal activity in the acute-phase serum only after the addition of rabbit serum as a source of exogenous complement (R. Gold and R. H. McLean, Abstr. 698, Pediatr. Res. 12:480, 1978).

Our proband, a young black male, was discovered because his serum totally lacked CH_{50} hemolytic activity and bactericidal function. Detailed functional analysis of serum complement components revealed selective absence of C6 activity. In addition, C6 was undetectable by radial immunodiffusion against monospecific antisera, providing evidence against the presence of a nonfunctional C6 molecule in his serum, and no evidence for a C6 inhibitor could be demonstrated.

The absence of C6 did not appear to affect many of the biological functions mediated by the complement cascade, including generation of opsonic and chemotactic activity. In addition, the coagulation mechanisms studied were normal. Bactericidal and hemolytic activities in the sera were restored by the addition of purified C6.

The absence of C6 activity in our patient appears to be caused by the inheritance of two autosomal codominant alleles, each defective in coding for C6 synthesis. A similar inheritance has been noted in other reports (8, 10) and has been postulated also for transmission of C6 deficiency in rabbits (7). Genetic analysis of the

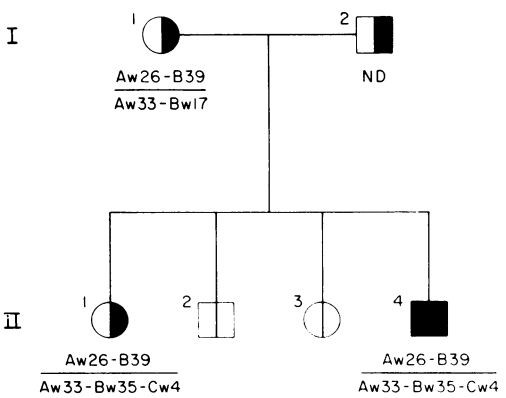

FIG. 2. *Family pedigree of the C6-deficient patient. The proband is II-4. Symbols: ◑, heterozygous for C6 deficiency; ■, homozygous for C6 deficiency; ◫, C6 not tested; A, HLA locus A; B, HLA locus B; w, HLA type.*

family of the proband indicated that the genes controlling C6 synthesis did not segregate with HLA alleles, and the assignment of heterozygosity in the parents and one sibling is tentative. The propositus, who is homozygous for C6 deficiency genes, was heterozygous for HLA haplotypes, and an HLA identical sibling was probably heterozygous for C6 deficiency. Only genes controlling the synthesis of complement components C2, C4, and factor B in the bypass pathway of complement activation have been mapped near the HLA region on the short arm of chromosome number 6 in man (6). Glass et al. (3), in a study of C6 electrophoretic variance in four C6-deficient families, have concluded that the C6 deficiency state is determined by a silent or null (C6d) allele at the same genetic locus as that determining structural (electrophoretic) variance. A previous report has also shown no evidence for linkage of C6 and HLA as well as a variety of other markers (13). The present report confirms these conclusions regarding linkage and extends the possible clinical and biological implications of the C6 deficiency state in man.

The recent observation that patients with deficiencies of the terminal components of the complement cascade (C6, C7, and C8) have a probable predisposition for bacteremic *Neisseria* infections rather than a variety of bacterial diseases is of particular interest. These individuals possess normal complement-mediated opsonization and chemotaxis but lack bactericidal activity (8–10, 15). Previous reports of C6 deficiency have been associated with repeated episodes of *N. meningitidis* infections and recurrent bouts of disseminated gonococcal infection but no history of other recurrent infections (8, 10). Responsibility of C6 deficiency for these recurrent episodes of bacteremic *Neisseria* infections can only be inferred but is strongly suggested. In our case of C6 deficiency, antimeningococcal bactericidal antibodies against the homologous strain were present in the acute serum. The absence of C6 appeared to be the significant defect since bactericidal function was restored with the addition of the complement donor serum (C) or pure C6. The presence of a nonfunctional bactericidal but normal opsonizing system in the acute-phase serum of a patient with bacteremic *Neisseria* infection supports the concept that serum bactericidal function is an important protective mechanism against *N. meningitidis* bacteremia (4).

The prevalence of complement component deficiencies in the select group of patients with either *N. meningitidis* and *N. gonorrhoeae* bacteremia is not yet known, but we have recently found another C8-deficient patient presenting with a history of recurrent episodes of meningococcal meningitis (M. Veeder et al., unpublished observations). Absence of detectable serum and secretory IgA in this patient raises further interesting speculation since *N. meningitidis* infections appear to be initiated at mucosal surfaces. We have thus identified one case each of C6, C7, and C8 deficiency among a group of 32 patients with systemic *Neisseria* infection tested for bactericidal or total hemolytic activity (CH_{50}) or both. Screening studies of apparently normal military recruits for homozygous inborn errors in the complement system by the 50% hemolytic complement assay demonstrated an incidence of 0.03% (14 per 41,082 persons studied) (5). A reasonable estimation of the prevalence of complement deficiencies in the select group of patients with bacteremic *Neisseria* infections is approximately 5 to 10% (or greater than 100 times the frequency in the normal population). More than 50% of all cases of late-acting complement component deficiencies (C6, C7, or C8) have been associated with bacteremic *N. meningitidis* or *N. gonorrhoeae* infections (Petersen et al., in press). Deficiency of C6, C7, or C8 probably represents a relatively uncommon risk factor, but assessment of the integrity of the complement system with the CH_{50} assay in patients with bacteremic *Neisseria* infections (especially in those with recurrent episodes) seems warranted.

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