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Bactericidal Screening Test for Late Complement Component Deficiencies or Defects

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Congenital complement deficiency has been described in disseminated *Neisseria* infections. Its occurrences in humans with other kinds of infections have not been described. In the past, CH50 determinations have been used to detect these deficiencies, but this procedure is time consuming and cumbersome. A method of determining the presence of late component deficiencies or defects is described which is easy and inexpensive to perform. An agar pour plate with a serum-sensitive *Escherichia coli* strain is made, and 2.5-mm wells are put in the agar. Unknown fresh sera are used to fill the wells. An absence of a zone of bacterial growth inhibition around the well after incubation at 37°C overnight indicates a late component defect or deficiency in the test serum. By applying this assay to 35 selected patients, four deficient patients were identified. One had a congenital C5 deficiency and three had C6 deficiencies. It is suggested that the assay be used as a screening test to study the relationship between congenital complement deficiencies and various kinds of infections, especially those caused by organisms which are partially serum sensitive.

Complement component deficiencies have been described recently in a variety of diseases, including systemic lupus erythematosus (3), hereditary angioedema (7), disseminated gonorrhea infections (5), and *Neisseria meningitidis* meningitis (3). The standard test to demonstrate whether the complement system is intact is the CH50 test (2). This involves diluting serum samples and determining the percentage of erythrocytes that each dilution is capable of lysing. This procedure is much too cumbersome to serve as a screening test for complement deficiencies. Because of an apparent association of inherited complement deficiency with *Neisseria* infections, it may be necessary to screen patients with such infections for deficiencies. The following report describes a rapid, efficient, and readily available method for detecting late complement component deficiencies.

MATERIALS AND METHODS

A serum-sensitive *Escherichia coli* which was susceptible to all the antibiotics tested, including ampicillin, was isolated from a patient. This organism was grown in increasing concentrations of nalidixic acid until it showed no zone of inhibition around a 30-μg nalidixic acid antibiotic disk from BBL Microbiology Systems. Then this organism was grown with a nalidixic acid-susceptible *Klebsiella pneumoniae* which contained an R-factor mediating resistance to many antibiotics. Transfer of resistance factor to the serum-sensitive *E. coli* was detected by growth on an agar plate containing 4 μg of ampicillin per ml and 30 μg of nalidixic acid per ml. The resultant organism was serum sensitive and resistant to many antibiotics including penicillin, ampicillin, carbenicillin, cefamandoe, kanamycin, and gentamicin. However, it was susceptible to chloramphenicol, amikacin, tetracycline, and trimethoprim-sulfamethoxazole by disk diffusion test.

Tube dilution susceptibility tests were performed on the resultant *E. coli* against penicillin, ampicillin, carbenicillin, and chloramphenicol, the antibodies usually employed to treat bacterial meningitis (9).

A 10-ml Trypticase soy agar plate (100 by 150 mm; BBL Microbiology Systems) was made containing 0.5 ml of *E. coli* which had been adjusted to a turbidity of 5 on the McFarland standard. When cool, 2.5-mm round reservoir wells were were made in the solidified agar. These wells were filled by micropipettes with the test sera. Overfilling had little effect on the results as long as the sample did not run into another well. Control sera with intact complement and serum inactivated at 56°C for 30 min were similarly placed into wells. The plate was incubated at 37°C until there was sufficient *E. coli* growth to cause turbidity in the agar. A clear zone around the well was interpreted as indicating intact late complement components in the test serum. The zone size can be quantitated by direct ruler measurement or by a Lily-Fisher antibiotics zone reader. The unused pour plates can be stored at 4°C for a minimum of 1 week without affecting the results.

To confirm the validity of the bactericidal assay, each serum specimen was also tested by a hemolytic plate assay with sensitized sheep erythrocytes by the method of Fong and Renaud (1).

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The sera found to be deficient by the bactericidal assay were sent to a complement laboratory, either to Celso Bianco at Downstate Medical Center or to Chester Alper at the Center for Blood Research in Boston. The components deficient or defective were determined by showing the restoration of hemolytic activity by the addition of a specific complement component to the test serum.

RESULTS

With the exception of chloramphenicol, the usual antibiotics administered for bacterial meningitis, including penicillin, ampicillin, carbenicillin, and gentamicin, did not affect the results of the test. Figure 1 shows that no zone of inhibition was present when 8 μg of gentamicin, 50 μg of ampicillin, and 100 μg of carbenicillin per ml were added to the wells in the concentrations usually achieved in the serum. The minimum inhibitory concentrations of antibiotics for this *E. coli* were greater than 200 μg/ml for carbenicillin, penicillin, and ampicillin, greater than 50 μg/ml for gentamicin, and 12 μg/ml for chloramphenicol.

The zone size can be used to approximate the percentage of normal total bactericidal complement in any given test serum sample. Figures 2 and 3 show that with dilutions of normal pooled human serum, there was a predictable decrease in the size of the zone of inhibition.

When fresh normal serum which has been inactivated at 56°C for 30 min was used, or when C3-deficient serum was used, no zone of inhibition was seen (Fig. 1). When completely C2-deficient serum was used, a normal size zone of inhibition was produced, indicating that when only the alternative pathway activation is present, a normal amount of bactericidal activity can be achieved.

The above assay was applied to fresh sera or freshly thawed sera stored at −70°C from 35 patients; most patients had bacterial meningitis,

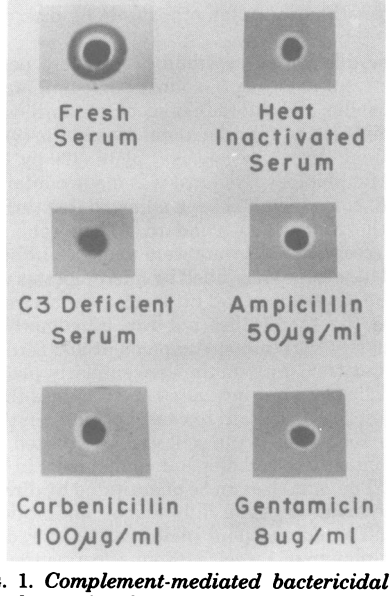

FIG. 1. *Complement-mediated bactericidal activity on the strain of E. coli used. Heated serum, C3-deficient serum, ampicillin, carbenicillin, and gentamicin showed no activity.*

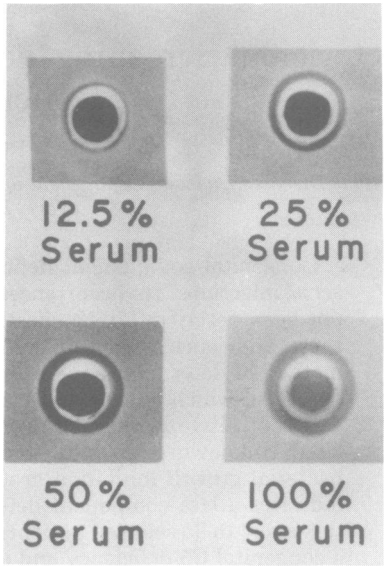

FIG. 2. *Zone size of inhibition with varying concentrations of serum (complement).*

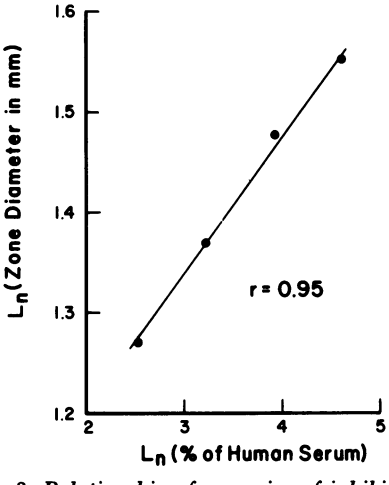

FIG. 3. *Relationship of zone size of inhibition to the concentration of fresh human serum (complement).*

but sera from patients with a variety of infections were included. Thirty patients' sera were found to have a normal bactericidal activity, including a patient with common variable immunoglobulin deficiency and two patients with C3b inactivator deficiency. Five patients showed no bactericidal activity, and the hemolytic complement assay confirmed the absence of complete complement activity. Four of the five were later shown to have specific congenital complement component deficiencies: three with C6 deficiency and one with a C5 deficiency. The remaining patient whose serum had no bactericidal activity had disseminated coagulopathy with very low C4, C3, and C6 levels. There were no false-positive or false-negative results in this small series (Table 1).

DISCUSSION

Complement has long been known to have bactericidal activity. Its role in protecting humans from bacterial infections varies with the species and strain of bacteria (7). Many of the enteric gram-negative bacteria are serum sensitive, and presumably complement-mediated bactericidal activity can protect the host from such organisms (6). Many strains of *Neisseria* are sensitive to human serum (8). Recent evidence has linked complement deficiencies to recurrent or bacteremia-causing *Neisseria* infections (5). It may be necessary to screen patients with these infections and possibly other populations to determine the prevalence of inherited complement deficiencies and its significance in this and other infections.

TABLE 1. *Results of complement assay*

Patient no.	Pathogen	Diagnosis	Bactericidal complement	Hemolytic complement
1	*Neisseria gonorrhoeae*	Arthritis-dermatitis syndrome	+	+
2	*Streptococcus pneumoniae*	Pneumonia; common variable immunoglobulin deficiency	+	+
3	*Neisseria meningitidis*	Meningitis	−	−
4	*N. meningitidis*	Meningitis; C3bINA deficiency	+	+
5	None	Meningitis; C6 deficiency	−	−
6	*N. meningitidis*	Meningitis; C5 deficiency	−	−
7	*N. meningitidis*	Meningitis; disseminated coagulopathy	−	−
8	*Haemophilus influenzae*	Meningitis	+	+
9	*N. meningitidis*	Meningitis; disseminated coagulopathy	+	+
10	*N. meningitidis*	Meningitis	+	+
11	*N. meningitidis*	Meningitis	+	+
12	*N. meningitidis*	Meningitis	+	+
13	*N. meningitidis*	Meningitis	+	+
14	*H. influenzae*	Meningitis	+	+
15	*N. meningitidis*	Meningitis; cribiform plate fracture	+	+
16	*S. pneumoniae*	Pneumonia	+	+
17	*S. pneumoniae*	Pneumonia; partial C2 deficiency	+	+
18	*H. influenzae*	Pneumonia; bacteremia	+	+
19	*S. pneumoniae*	Pneumonia	+	+
20	Herpes-zoster	Varicella	+	+
21	None	Recurrent urticaria	+	+
22	None	Asymptomatic C3bINA deficiency	+	+
23	*Salmonella typhi*	Typhoid; peritonitis	+	+
24	*S. pneumoniae*	Pneumonia	+	+
25	*N. gonorrhoeae*	Arthritis-dermatitis syndrome	+	+
26	Respiratory virus	Upper respiratory infection	+	+
27	*Staphylococcus aureus*	Bacteremia; nephrotic syndrome	+	+
28	*Salmonella typhimurium*	Sickle cell disease; osteomyelitis	+	+
29	*Salmonella enteritidis*	Gastroenteritis; bacteremia	+	+
30	*Klebsiella pneumoniae*	Partial DiGeorge syndrome	+	+
31	*H. influenzae*	Subcutaneous abcesses	+	+
32	*H. influenzae*	Epiglottitis; bacteremia	+	+
33	*N. meningitidis*	Meningitis; C6 deficiency	−	−
34	*N. meningitidis*	Meningitis	+	+
35	*Flavobacterium meningosepticum*	Meningitis	+	+

It has been presumed that hemolytic complement involves the same components and mechanisms as bactericidal complement. The behavior of the two kinds of serum activity is identical, as shown above. Both require C3, both are active in the absence of C2, both are inactivated at 56°C for 30 min, and both require late components for complete action (shown for C5 and C6 above). The interaction of complement with bacteria has not been studied as extensively as with erythrocytes. In dealing with infectious diseases, it is more realistic to test the ability of a patient's serum to kill a bacterium than its ability to lyse an erythrocyte, although no differences in the mechanism of activation or membrane destruction have been observed.

The assay described has the advantage that it can be done in any infectious diseases or microbiology laboratory with minimal cost. However, it cannot be used to detect alternative pathway defects unless there is a concomitant late component defect of C5 to C8. Fortunately, alternative pathway defects are much rarer.

Serum with complete absence of antibodies is expected to give the same results on this test as serum with complete absence of C2: a normal bactericidal level.

In this series of 35 patients tested, 5 were found to have deficient bactericidal activity. Four of the five had congenital late component deficiencies with normal levels of C3. If only C3 were assayed as is done in most hospitals, their deficiencies would not have been detected. Five deficiencies of 35 is high and reflects the select patient population tested with many who had recovered from bacterial meningitis, usually caused by meningococci. This supports the association noted by Petersen et al. (5).

The incidence of congenital late component deficiencies in patients suffering from other kinds of infections is unknown. Hopefully, the availability of an easy and accessible assay such as the one proposed will permit better definition of the role of complement in human host defenses against various organisms.

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