INFECTION AND IMMUNITY, Oct. 1977, p. 14-22

Vol. 18, No. 1
Printed in U.S.A.

Complement Levels in Pneumococcal Pneumonia

J. DONALD COONROD* AND BARBARA RYLKO-BAUER

Veterans Administration Hospital and the Division of Infectious Diseases, Department of Medicine, University of Kentucky School of Medicine, Lexington, Kentucky 40506*

Received for publication 2 May 1977

Levels of complement proteins and functional activity of the alternate complement pathway were assessed in 39 patients with pneumococcal pneumonia. Mean levels of C3 and properdin and the functional activity of the alternate pathway in acute sera were significantly ($P < 0.05$) below normal, whereas levels of components of the early classical pathway were normal. Although levels of factor B were in the normal range, they correlated significantly with C3 levels; there was no significant relation between C3 levels and C4 or C1q levels. The 19 patients with pneumococcal pneumonia and bacteremia had significantly lower mean values of properdin and factor B than the 20 patients without bacteremia, suggesting a more severe depression of the alternate complement pathway with bacteremia. During convalescence, complement levels were normal or elevated in most of the patients, but mean levels of properdin remained significantly below normal in bacteremic patients. Functional activity of the alternate pathway also remained below normal. These results indicate that there is a selective depression of the alternate pathway in patients with pneumococcal pneumonia, and they are consistent with the concept that the alternate pathway has an important role in host defenses in pneumococcal infection.

Both the classical and alternate (properdin) complement pathways have been shown to participate in opsonization of pneumococci (9, 26). The alternate pathway may be the more important determinant of natural immunity to pneumococci in humans, but comparative studies of the two pathways in naturally occurring pneumococcal infection have been few. Reed et al. (16) demonstrated recently that levels of factor B, a component of the alternate pathway, were below normal in patients with pneumococcal infections, whereas levels of components of the early classical pathway (C1q and C4) were within normal limits. These findings suggested a selective alteration of the alternate complement pathway in patients with pneumococcal infection. In the present studies, complement components of the classical and alternate pathways and functional activity of the alternate pathway were measured in patients with pneumococcal pneumonia. The results showed a selective depression of the alternate pathway during the acute phase of the infection, with the depression being more marked in patients with pneumonia accompanied by pneumococcal bacteremia.

MATERIALS AND METHODS

Patients. Thirty-nine patients who were admitted to the hospital with fever, purulent sputum, and a clinical history suggestive of acute bacterial pneumonia were studied. All had roentgenographic evidence of pulmonary consolidation. Cultures of blood and sputum were obtained before antibiotic therapy, and *Streptococcus pneumoniae* was isolated from cultures of the blood of 19 cases, in pure culture from transtracheal aspirates of 14 cases, and as the predominant organism from expectorated sputum of 6 cases. The latter six patients showed clinical improvement within 72 h on low doses of penicillin G. The mean age of the subjects was 56.8 years (range, 25 to 80 years). Thirty-one of the patients were males; there were 32 whites and 7 blacks. Thirteen of the patients were considered by their physicians to be alcoholics, and 11 subjects had clinical and laboratory evidence of chronic obstructive airways disease. None of the patients had documented collagen-vascular disease, neoplastic disease, cirrhosis, or sickle cell disease. Acute sera were obtained within 48 h after admission to the hospital. Convalescent sera were obtained 7 to 21 days after admission (mean, 13.5 days), except from three patients who died and from three individuals who were lost to follow-up. Blood samples were allowed to clot at room temperature for 30 to 60 min, and the serum was stored in multiple small samples at −70°C.

Complement proteins. Levels of complement proteins were measured by a modification of the radial immunodiffusion method of Mancini et al. (12). Calibration curves were prepared with dilutions of reference standards for all determinations, and internal controls were included where appropriate. The diameters of precipitin rings were measured to the nearest 0.1 mm with a magnifying (×3.5) ocular and an illuminated viewer. Properdin levels were determined with an optimal dilution (1:50) of anti-properdin serum

incorporated into agarose (0.6%) in 0.01 M phosphate-buffered saline (pH 7.0) with 0.01 M ethylenediaminetetraacetate (EDTA) and 0.1% sodium azide. Wells of 3-mm diameter were filled with 20 μl of serum. Precipitin reactions were maximal after incubation for 48 h at 37°C in a moist box. The antiproperdin serum (kindly provided by Ann B. Bjornson, University of Cincinnati College of Medicine) was produced with human properdin purified by the method of Pensky et al. (14). The antiserum gave a single precipitin line in immunodiffusion with human serum and gave a reaction of identity against properdin and an antiproperdin serum provided originally by Clark West (University of Cincinnati). C1q and C5 levels were determined with an optimal dilution (1:40 and 1:15, respectively) of antiserum incorporated into 1% agarose in Veronal buffer (pH 8.6) and 0.04 M EDTA. Wells of 3-mm diameter were used, and precipitin reactions were developed for 24 h at room temperature. Antisera to C1q and C5 were obtained from Behring Diagnostics (Somerville, N.J.) and gave a single precipitin line with human serum. Levels of properdin, C1q, and C5 were expressed as percentages of standard reference sera. C3, C4, and factor B levels were measured with commercially available immunoplates and reference standards, obtained, respectively, from Hyland Laboratories (Costa Mesa, Calif.), Meloy (Springfield, Va.), and Behring Diagnostics. Precipitin reactions for these components were developed for 24 h at room temperature. In some cases, serum C3 levels were measured by radial immunodiffusion with antiserum, which was specific for the B-antigenic determinant of the native C3 molecule. This antiserum (kindly provided by Ann Bjornson) was prepared according to the method of West et al. (23); it gave a single precipitin line against fresh normal serum but no reaction with aged human serum.

Normal levels of complement proteins were established with sera from 30 adults (mean age, 51.3 years, and range, 19 to 65 years; there were 26 whites and 4 blacks).

Immunoelectrophoresis. Electrophoresis of serum C3 and factor B was performed with 1.5% Noble agar (Difco, Detroit, Mich.) in 0.02 M EDTA and Veronal buffer (pH 8.2; ionic strength, 0.05). Electrophoresis was conducted at 200 V for 90 min, and precipitin reactions were obtained with antiserum to C3 (Hyland Laboratories) or factor B (Behring Diagnostics). Controls consisted of fresh and aged normal human serum.

Hemolytic complement. Serum hemolytic complement levels were measured as 50% hemolytic units (CH_{50}) by a spectrophotometric method, with a reaction volume of 4.0 ml (13). Normal levels were established with sera from 51 subjects (mean age, 48.3 years, and range, 19 to 65 years; 43 whites and 8 blacks).

Zymosan test. The functional activity of the alternative complement pathway was measured by the consumption of total hemolytic complement after zymosan activation. A modification of the method of Fine et al. (4) was used. Zymosan (Nutritional Biochemicals Corp., Cleveland, Ohio) in normal saline was boiled for 2 h, washed three times in normal saline, and brought to a final concentration of 2 mg/ml. A stock solution of 0.1 M ethyleneglycol-bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA) (Sigma Chemical Co., St. Louis, Mo.) and 0.02 M $MgCl_2$ was prepared in normal saline, with adjustment of the pH to 7.45 before dilution to final volume with saline. In the routine test, 1 ml of fresh serum was mixed with 0.1 ml of this buffer, and the mixture was incubated at room temperature for 5 min. The serum was divided into two (0.5-ml) aliquots; one aliquot was mixed with 0.5 ml of zymosan and the other aliquot, which served as the control, was mixed with 0.5 ml of normal saline. The samples were mixed and incubated at 37°C for 45 min. They were remixed once by inversion at the middle of the incubation period. Zymosan was removed by centrifugation at 2,500 × *g* for 10 min at 4°C. To recalcify, 0.05 ml of $CaCl_2$ (0.1 M) was added to each aliquot. The CH_{50} titer of the samples was determined, and the number of CH_{50} units consumed by zymosan was taken as the difference in CH_{50} content of the control and the zymosan-treated samples. This test for measurement of alternate pathway activity was validated by the following studies. The amount of spontaneous activation (or deterioration) of complement in saline control samples was determined by comparing the CH_{50} content of these samples with the CH_{50} content of the original sera. With 25 normal sera, the mean (± standard deviation [SD]) reduction in CH_{50} content due to spontaneous activation was 16.5 ± 5.9%. The efficacy of EGTA-chelated serum in blocking activation by the classical pathway was tested by using optimally sensitized sheep erythrocytes (2×10^9/ml) in the test in the same manner that zymosan was usually used. With five normal sera, the mean (±SD) reduction in CH_{50} content by sensitized sheep erythrocytes was 19.3 ± 6.5%. In contrast, with 51 normal sera, the mean (±SD) reduction in CH_{50} content by using zymosan in the test was 82.1 ± 18.9%.

Since Mg levels are an important determinant of zymosan-induced consumption of complement in EGTA-chelated serum, the effect of varying Mg levels in the test was determined with Mg-EGTA buffers of varying Mg content. Maximum activation by zymosan was achieved in Mg-EGTA buffers with a Mg content of 0.01 to 0.03 M. The Mg content (0.02 M) of the Mg-EGTA buffer routinely used in the test thus provided a twofold excess of Mg over the minimal amount needed for maximal complement activation. In additional studies, serum Mg levels were measured in 25 acute sera and 21 convalescent sera from patients with pneumococcal pneumonia. The assays were performed by atomic absorption by Joseph Boone at the University of Kentucky laboratories. Mg levels for all the sera fell within the normal range (0.0006 to 0.0013 M), and the mean levels of Mg in the acute sera (0.00094 M) and the convalescent sera (0.00095 M) were not significantly different from the normal mean value (0.00093 M).

The results obtained by zymosan activation in chelated serum were highly reproducible; six replicate samples tested on the same day for each of three individuals varied by less than 0.5% from the mean value, and results with sera tested on three different occasions during a 6-month period for five individuals showed less than 7% variation from the mean. Under the conditions of the assay, the amount of available

hemolytic complement that was consumed by zymosan in 51 normal sera correlated with the total complement content of the sera ($r = 0.76$; $P < 0.001$). Therefore, results obtained with patients' sera in the assay were compared with the results of normal sera containing the same level (±5%) of hemolytic complement. Where there were several normal sera of the same complement level, the results were averaged for use in the comparison.

Antigens. Soluble pneumococcal antigens were measured by counterimmunoelectrophoresis, with methods that have been described elsewhere (1).

Statistics. Probability values for differences in frequency were determined by Fisher's exact test. Linear regression coefficients were calculated with a Monroe model 1860 calculator. Complement levels were compared with the t statistic, with correction for differences in variances of groups (7). A probability value (P) of ≤ 0.05 was considered significant.

RESULTS

Complement levels. Hemolytic complement activity and levels of several proteins of the classical and alternate complement pathways were measured in acute sera from 39 patients with pneumococcal pneumonia. The results are shown in Table 1. Mean levels of serum C3 and properdin, an alternate pathway component, were significantly lower in patients than in normal controls. Hemolytic complement levels in the patients' acute sera were lower than normal, but not significantly so ($P < 0.1$). In convalescent sera from 33 of the 39 patients, mean levels of C1q, C3, and C5, as well as factor B, an alternate pathway component, were significantly higher than normal. Mean levels of hemolytic complement and all components except C4 and C5 were significantly higher in convalescent than in acute sera for the 33 patients with both values. C4 levels varied little during the course of the infection, and mean C5 levels were above normal in both acute and convalescent sera.

C3 levels were measured in 31 acute sera and 27 convalescent sera with antiserum that was specific for the B-antigenic determinant of the native C3 molecule. The results with antisera specific for B antigen were virtually identical with those obtained in the routine test for C3, and linear regression analysis showed a coefficient of correlation (r) between the two of 0.95. This finding suggested that C3 levels, as measured in the routine studies, were indicative of the levels of functionally active C3.

Immunoelectrophoresis of C3 and of factor B in 20 acute and 15 convalescent sera was carried out with the antisera that had been used for routine measurement of C3 and of factor B. Fresh and aged normal human sera served as controls. The precipitin reactions obtained with C3 and factor B from each of the patients' sera were identical with the results obtained with fresh normal human serum. None of the patients' sera contained detectable quantities of the characteristic determinants of C3 and factor B which were observed with aged human serum. These findings suggested that the C3 and factor B proteins measured in patients' sera were the native molecules and not inactive derivatives.

Alternate pathway activity. Functional activity of the alternate complement pathway was evaluated by measuring zymosan-induced consumption of hemolytic complement (CH_{50}) in sera treated with EGTA to block the classical complement pathway. Since the quantity of complement consumed by zymosan varied with the complement content of the sera, the results with patients' sera were compared with normal sera of the same total content of hemolytic complement. The functional activity of the alternate pathway was markedly depressed in the acute phase of pneumococcal pneumonia. Mean consumption of complement in acute sera from the

TABLE 1. *Serum complement levels in pneumococcal pneumonia*

Group	Complement component (mean ± SD)						
	C1q (% of reference standard)	C4 (mg/100 ml)	Properdin (% of reference standard)	Factor B (mg/100 ml)	C3 (mg/100 ml)	C5 (% of reference standard)	Hemolytic complement (units)
Normals	98.4 ± 9.2	36.5 ± 6.6	98.1 ± 12.6	20.9 ± 3.2	132.9 ± 20.6	98.4 ± 15.4	92.8 ± 17.6
Patients with pneumococcal pneumonia							
Acute ($n = 39$)	97.2 ± 27.1	39.2 ± 13.2	62.9 ± 22.0	19.7 ± 6.7	116.6 ± 34.4	107.4 ± 30.1	83.8 ± 17.8
Convalescent ($n = 33$)	107.5 ± 21.2	37.9 ± 9.2	90.9 ± 17.9	27.7 ± 7.8	161.9 ± 44.6	111.7 ± 22.0	99.4 ± 16.8
P value							
Acute vs normal[a]	NS[b]	NS	<0.001	NS	<0.02	NS	NS
Convalescent vs normal[a]	<0.05	NS	NS	<0.001	<0.005	<0.05	NS
Acute vs convalescent[c]	<0.01	NS	<0.001	<0.001	<0.001	NS	<0.005

[a] t test for independent variables.
[b] NS, Not significant ($P > 0.05$).
[c] Paired (dependent) t test for patients with both values.

39 patients with pneumonia was 63.0% of the consumption in matched normal human sera ($P < 0.001$). In convalescent sera from 24 of the 39 patients, functional activity of the alternate pathway was 84.9% of the normal value—a significant increase from the acute sera ($P < 0.001$) though still significantly below the normal level ($P < 0.05$).

Complement levels and bacteremia. To define the complement profile in pneumococcal pneumonia further, complement levels in patients with bacteremic pneumococcal pneumonia were determined and compared with the levels of cases of abacteremic pneumococcal pneumonia. Of the 39 patients in the study, 19 had pneumococcal bacteremia and 20 had sterile blood cultures. Levels of components of the classical pathway and of hemolytic complement of these two groups of patients are tabulated in Table 2. Mean levels of C3 and hemolytic complement in acute sera from bacteremic patients were significantly below normal, whereas the corresponding mean values for abacteremic patients did not differ significantly from normal. Levels of C5 and hemolytic complement for bacteremic patients were significantly below the corresponding values for abacteremic patients. C1q levels in bacteremic patients were lower than normal and lower than C1q for abacteremic patients, but these differences were not significant ($P < 0.20$). With convalescent sera, elevated levels of several of the classical pathway components and hemolytic complement were observed in both groups of patients, and there were no significant differences between the two groups.

Values for properdin levels are shown in Fig. 1. With acute sera, 16 of 19 bacteremic patients were more than 2 SDs below the normal mean, and 10 of 20 abacteremic patients also had subnormal values. Mean properdin levels of both groups were significantly below normal ($P < 0.001$). However, the mean properdin level (±SD) for bacteremic patients (52.8 ± 17.8%) was significantly below the mean properdin level

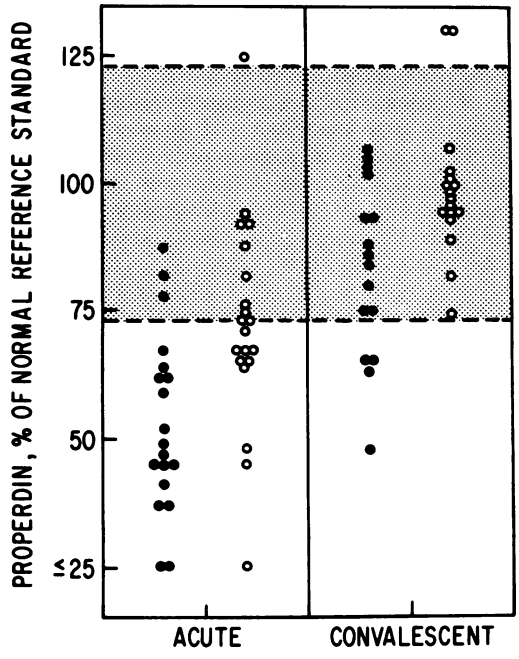

FIG. 1. *Serum levels of properdin in patients who had pneumococcal pneumonia with bacteremia* (●) *or without bacteremia* (○). *The shaded area represents the normal mean ± 2 SDs.*

TABLE 2. *Components of the classical complement pathway and hemolytic complement in bacteremic versus abacteremic pneumococcal pneumonia*

Group	Complement component (mean ± SD)[a]				
	C1q	C4	C3	C5	Hemolytic complement
Normals	98.4 ± 9.2	36.5 ± 6.6	132.9 ± 20.6	98.4 ± 15.4	92.8 ± 17.6
Pneumonia, acute serum					
Bacteremic ($n = 19$)	88.2 ± 27.1	33.0 ± 14.4	106.4 ± 28.6	97.7 ± 27.6	78.0 ± 19.2
Abacteremic ($n = 20$)	97.2 ± 22.6	39.3 ± 11.5	126.2 ± 37.2	117.0 ± 30.1	89.6 ± 15.2
P value[b]					
Bacteremic vs normal	NS[c]	NS	<0.005	NS	<0.02
Abacteremic vs normal	NS	NS	NS	<0.02	NS
Bacteremic vs abacteremic	NS	NS	NS	<0.05	<0.05
Pneumonia, convalescent serum					
Bacteremic ($n = 16$)	106.0 ± 25.5	37.8 ± 10.7	161.4 ± 32.7	109.9 ± 22.7	103.9 ± 17.2
Abacteremic ($n = 17$)	108.4 ± 16.6	38.0 ± 7.9	162.4 ± 54.0	113.5 ± 22.0	95.4 ± 16.3
P value[b]					
Bacteremic vs normal	NS	NS	<0.005	NS	<0.05
Abacteremic vs normal	<0.05	NS	<0.05	<0.02	NS
Bacteremic vs abacteremic	NS	NS	NS	NS	NS

[a] Units of measurement are as indicated in Table 1.
[b] *t* test for independent variables.
[c] NS, Not significant ($P > 0.05$).

for abacteremic patients (73.0 ± 21.5%) ($P < 0.005$). With convalescent sera (Fig. 1), properdin levels remained more than 2 SDs below normal in 4 of 16 bacteremic patients, whereas all abacteremic patients had levels in the normal range. The mean properdin level (±SD) in convalescent sera from bacteremic patients was 83.2 ± 17.8%. This level was significantly below normal ($P < 0.005$) and also lower than the mean level of convalescent sera from abacteremic patients (98.5 ± 14.8%) ($P < 0.02$).

Factor B levels are given in Fig. 2. The majority of patients in both groups had normal levels of factor B in acute sera. However, the mean level of factor B in acute sera from bacteremic patients (17.4 ± 6.3 mg/100 ml) was significantly below normal ($P < 0.05$) and significantly lower than the mean level for abacteremic patients (22.0 ± 6.5 mg/100 ml) ($P < 0.02$). During convalescence, mean factor B levels were elevated in both the bacteremic group (27.1 ± 6.2 mg/100 ml) and the abacteremic group (28.2 ± 7.5 mg/100 ml) ($P < 0.001$ for both groups).

Linear regression analysis showed that factor B levels in acute sera correlated significantly with C3 levels, and the values for bacteremic patients clustered in the lower range for both proteins (Fig. 3). Similar analyses of C1q and C4 with C3 levels failed to show any significant relationship ($r = 0.19$ for C3 and C1q; $r = 0.25$ for C3 and C4).

Results of studies of the functional activity of the alternate pathway for bacteremic and abacteremic patients are shown in Fig. 4. Both groups of patients had depressed alternate pathway activity in acute sera as compared with matched normal subjects ($P < 0.001$). The depression was more pronounced in bacteremic patients; these individuals averaged 57.9% of the mean complement consumption achieved by normal subjects, whereas the comparable figure for abacteremic patients was 68.2% of normal. However, the difference between the two groups of patients was not statistically significant. During convalescence, alternate pathway activity was within the normal range for most patients in both groups. For bacteremic subjects, complement consumption by zymosan in the convalescent sera averaged 81.0% of the mean value for normals, and abacteremic patients averaged 88.1% of the normal value. Neither group of patients differed significantly from normal.

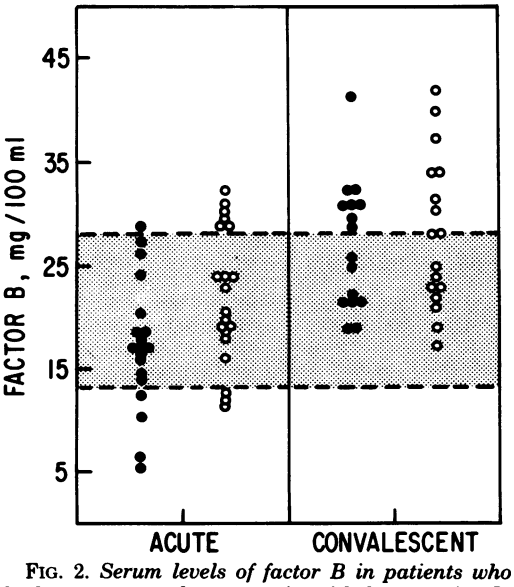

FIG. 2. *Serum levels of factor B in patients who had pneumococcal pneumonia with bacteremia (●) or without bacteremia (○). The shaded area represents the normal mean ± 2 SDs.*

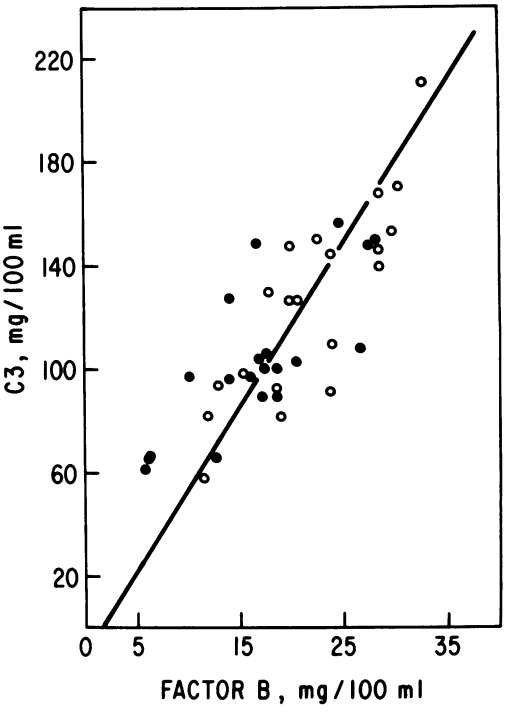

FIG. 3. *Correlation of serum C3 and factor B levels in acute sera of 19 patients who had pneumococcal pneumonia with bacteremia (●) and 20 patients who had pneumococcal pneumonia without bacteremia (○). The coefficient of correlation (r) was 0.79, as determined by linear regression analysis ($P < 0.001$).*

Clinical features. Clinical characteristics of patients with bacteremic or abacteremic pneumococcal pneumonia were compared in an effort to gain insight into the reasons for differences in their complement levels (Table 3). The two groups did not differ significantly in age, race, or sex, but bacteremic patients did appear

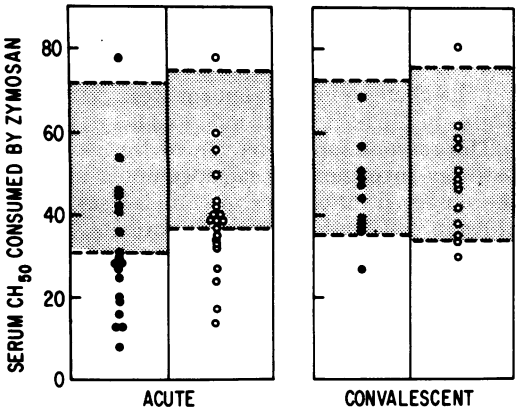

FIG. 4. *Consumption of hemolytic complement (CH_{50}) by zymosan in sera from patients who had pneumococcal pneumonia with bacteremia (●) or without bacteremia (○). The shaded areas represent the means ± 2 SDs for normal subjects whose total hemolytic complement levels matched those of the patients.*

TABLE 3. *Clinical and laboratory characteristics of patients who had pneumococcal pneumonia with or without bacteremia*

Patients	Blood culture		*P* value
	Positive	Negative	
Total (no.)	19	20	
Mean age (yrs)	55.2	58.4	NS[a]
Males (no.)	18	13	NS
Caucasians (no.)	14	18	NS
Admission granulocyte count:			
Mean × $10^3/mm^3$	9.2	16.8	< 0.005
Count < $4,000/mm^3$ (no.)	6	0	0.05
Pulmonary findings:			
Multilobar pneumonia (no.)	9	7	NS
Pleural fluid (no.)	6	0	0.05
Hospital course:			
Mean days of fever, >37.8°C[b]	5.0	2.5	< 0.05
Mean days in the hospital[b]	17.1	8.8	< 0.005
Deaths (no.)	3	0	NS
Associated diseases (no.)			
Chronic alcoholism	10	3	0.05
Chronic obstructive airways disease	4	7	NS
Diabetes millitus	2	2	NS
Others[c]	3	4	NS

[a] NS, Not significant ($P > 0.05$).
[b] Deceased patients excluded.
[c] Includes peptic ulcer disease, atherosclerotic heart disease, and essential hypertension.

to have a more severe illness. There were significantly more pleural effusions in bacteremic patients, as judged by roentgenograms obtained in the lateral decubitus position, and three patients in the bacteremic group had documented pleural empyema. Fever persisted longer in bacteremic patients, and they had a significantly longer stay in the hospital. The only three deaths in the study occurred in patients with bacteremia.

Both groups of patients had similar underlying illnesses, with the exception that significantly more bacteremic patients were considered to be alcoholics by their physicians. However, as indicated in Table 4, mean complement levels for 10 alcoholic patients with pneumococcal bacteremia did not differ significantly from mean complement levels of 9 bacteremic patients who were not alcoholics.

Pneumococcal serotypes. The possibility of a relationship between the serotype of the infecting pneumococcus and complement levels was considered. Seven of the 39 patients in the study were infected with type 3 pneumococci; four cases were due to type 4, and three cases each were caused by types 6, 19, and 23. There were 14 different pneumococcal types associated with the remaining 19 cases. The number of patients infected with any given serotype was too small to warrant statistical analysis of the results. However, it was apparent that altered complement levels were associated with pneumonia caused by any one of a variety of pneumococcal serotypes.

Capsular polysaccharide antigenemia. Complement levels were evaluated in relation to capsular polysaccharide antigenemia. Capsular polysaccharide was detected by counterimmunoelectrophoresis in acute sera from 11 of the 39 patients. Since each of these 11 patients was also bacteremic, complement levels could not be evaluated independently of bacteremia. The antigenemic patients were therefore compared with the eight bacteremic patients who were not antigenemic. Hemolytic complement and mean levels of C1q, C3, C4, C5, and factor B were not significantly different in the two groups. The

TABLE 4. *Complement parameters for acute sera of alcoholic or nonalcoholic subjects with bacteremic pneumococcal pneumonia*

Complement parameter	Mean ± SD[a]	
	Alcoholic ($n = 10$)	Not alcoholic ($n = 9$)
C1q	93.0 ± 24.6	82.3 ± 30.5
C4	27.6 ± 8.3	39.1 ± 17.6
Properdin	55.1 ± 16.6	50.2 ± 21.0
Factor B	16.3 ± 5.7	18.6 ± 7.1
C3	105.2 ± 26.2	107.8 ± 32.6
C5	93.9 ± 26.1	102.5 ± 30.5
Hemolytic complement	76.5 ± 16.5	79.7 ± 22.6
CH_{50} consumed by zymosan	56.4 ± 19.2	60.1 ± 14.2

[a] Units of measurement are as indicated in Table 1. Alcoholic and nonalcoholic subjects did not differ significantly in any complement parameter ($P > 0.05$).

mean properdin level of antigenemic patients was 45.4% of normal, whereas the corresponding value of non-antigenemic patients was 62.0% of normal; mean consumption of hemolytic complement by zymosan was lower in the sera of antigenemic patients (48.7% of normal) than of non-antigenemic individuals (76.6% of normal). However, neither of these differences was significant ($P < 0.10$).

DISCUSSION

Our studies indicate that there are abnormalities of several components of complement in patients with pneumococcal pneumonia. Acute sera from 39 patients showed a significant depression of mean levels of C3, which is a pivotal complement component for activation of the terminal complement sequence by either the classical or alternate complement pathway. In studies of the alternate pathway, mean levels of properdin were significantly decreased, and functional activity of the alternate complement pathway, as measured by zymosan stimulation, was also markedly depressed. Although levels of factor B were in the normal range, they correlated significantly with C3 levels, suggesting a common determinant for levels of C3 and factor B. In contrast, studies of the early classical pathway showed normal mean levels of C1q and C4, and levels of these proteins did not correlate significantly with C3 levels. Total hemolytic complement in acute sera was at the lower limit of normal, a finding which could have reflected lowered levels of C3 or changes in levels of other terminal components of the classical pathway. Taken together, these findings strongly suggest that there is a selective depression of the alternate complement pathway in patients with pneumococcal pneumonia. These observations are consistent with the early report of Hinz (8), who noted diminished properdin activity in cases of pneumococcal pneumonia, and with the recent observations of Reed et al. (16), who observed decreased C3 and factor B levels, with normal C1q and C4 levels, in patients with acute pneumococcal infections.

In extending these observations, we compared complement levels of the patients with pneumonia who had an accompanying pneumococcal bacteremia with those who were abacteremic. Properdin levels and functional activity of the alternate pathway were below normal in both groups of patients, but properdin levels were significantly lower in the bacteremic patients. In addition, bacteremic patients had lower levels of factor B, C5, and hemolytic complement than the abacteremic subjects. C1q and C4 levels did not differ significantly in the two groups. There was, therefore, evidence of selective depression of the alternate complement pathway in both bacteremic and abacteremic patients, but the changes in complement were greater in degree in the bacteremic individuals.

The reasons for depression of the alternate pathway in pneumococcal pneumonia and for the observed differences in complement levels of bacteremic and abacteremic patients are not clear. One possible explanation for the findings may be a selective activation and consumption of alternate pathway proteins in pneumococcal pneumonia. In varying degrees, most serotypes of pneumococci are able to activate the alternate pathway in vitro (24), and it is by this process that opsonically active C3b is thought to be fixed to pneumococci. Conceivably, the extent of consumption of complement by pneumococci in patients with pneumonia could depend on the concentration of organisms in various tissues and on whether organisms were present in the circulation. In vitro, more than 10^5 pneumococci per ml are needed to consume measurable amounts of complement by the alternate pathway (3). This level of organisms may be attained readily in the lung, but there are seldom more than a few hundred pneumococci per milliliter in the circulation in pneumococcal pneumonia (5, 21). The effects of a sustained bacteremia at this level, as may occur in pneumococcal pneumonia, are not known. Robertson et al. (17) in studies of pneumococcal pneumonia in dogs concluded that there was a loss of bactericidal activity for pneumococci in the blood after the development of pneumococcal bacteremia; cessation of bacteremia was accompanied by an increase in the bactericidal activity of the blood. Unfortunately, in these early studies the specific serum factors responsible for the bactericidal capacity of the serum were not defined, and we can only surmise that complement was a factor. In the present studies, patients with bacteremia had evidence of a more severe infection than did abacteremic patients, as has been noted by a number of investigators in both the pre- and postantibiotic eras (21, 22). If increased utilization of complement were a major determinant of complement levels in pneumococcal pneumonia, it seems possible that the extent of the infection could have influenced the amount of complement consumed. However, it is not certain that the reduction in complement levels we observed was due to increased consumption of complement. Low complement levels in several disease states have been shown to be due to increased complement consumption, but fractional catabolic rates must be determined to prove hypercatabolism.

Patients who develop pneumococcal pneu-

monia could have some impairment of the alternate pathway antedating the infection. There are precedents for this possibility. It is established, for example, that individuals with sickle cell disease fail to fix the essential C3 opsonin to pneumococci to a normal degree (25). This failure is due to reduced functional activity of the alternate pathway (10), which may be caused by a deficiency of C3 proactivator convertase (alternate pathway factor D) (11). Individuals with sickle cell disease are especially susceptible to severe infections, and particularly to pneumococcal meningitis (18, 20). If there were preexisting abnormalities of the alternate pathway in the patients we studied, these abnormalities might have predisposed to the development of pneumococcal pneumonia or of pneumococcal bacteremia. This possibility cannot be excluded by the present results. Studies of complement levels in convalescent sera did establish that most of the patients were able to increase complement levels in the normal range as the infection subsided. Mean levels of factor B, properdin, and C3, and the functional activity of the alternate pathway all increased significantly between the acute and convalescent phases of infection, and factor B and C3 were significantly above the normal range during convalescence. These latter changes could reflect the function of factor B and C3 as so-called acute-phase reactants (19) and do not necessarily indicate that the patients had normal levels of the components at the time the infection was acquired. Mean properdin levels returned to the normal range for the pneumonia patients as a whole during convalescence, but the mean level for bacteremic patients remained significantly below normal during the period in which the patients were observed. In addition, mean functional activity of the alternate pathway was slightly below normal during convalescence. On the basis of these findings we cannot exclude a preexisting abnormality of complement in at least some of the patients.

We attempted to exclude from the study patients who had diseases known to be associated with decreased complement levels. There were, however, 13 patients who were considered by their physicians to be alcoholics. Alcoholism, when complicated by hepatic cirrhosis, has been associated with depressed levels of C3, factor B, C4, C5, and hemolytic complement (2, 6, 15). None of the patients included in the present study had clinical or laboratory evidence of cirrhosis, but an attempt was made to evaluate the possible effects of alcoholism on complement levels. Alcoholic and nonalcoholic subjects with bacteremic pneumococcal bacteremia were compared. No significant differences between these two groups were found, suggesting that alcoholism per se was not a major determinant of the low complement levels seen in bacteremic individuals.

Activation of complement by pneumococci seems to be initiated principally by antigens in the cell wall, but certain type-specific capsular polysaccharides can also activate the alternate pathway. Capsular polysaccharide is occasionally present in the circulation in pneumococcal pneumonia, but the concentration of circulating antigen is usually much lower than the quantity of antigen required for activation of complement in vitro (24). In the present study, complement levels in patients with antigenemia did not differ significantly from the complement levels of non-antigenemic patients. Properdin levels and functional activity of the alternate pathway were lower in antigenemic than in non-antigenemic individuals, but these differences were not statistically significant ($P < 0.10$).

ACKNOWLEDGMENTS

This work was supported by the Medical Research Service of the Veterans Administration.

We are indebted to David Drennan for assistance in collecting the clinical samples and to Peter Walzer and Ward Bullock for reviewing the manuscript. We also thank Ann B. Bjornson for helpful discussions of the work and Grant W. Somes for help with the statistics.

LITERATURE CITED

1. **Coonrod, J. D., and D. P. Drennan.** 1976. Pneumococcal pneumonia: capsular polysaccharide antigenemia and antibody responses. Ann. Intern. Med. **84**:254–260.
2. **DeMeo, A. N., and B. R. Anderson.** 1972. Defective chemotaxis associated with a serum inhibitor in cirrhotic patients. N. Engl. J. Med. **286**:735–740.
3. **Fine, D. P.** 1975. Pneumococcal type-associated variability in alternate complement pathway activation. Infect. Immun. **12**:772–778.
4. **Fine, D. P., S. R. Marney, Jr., D. G. Colley, J. S. Sergent, and R. M. DesPrez.** 1972. C3 shunt activation in human serum chelated with EGTA. J. Immunol. **109**:807–809.
5. **Finland, M., and W. C. Spring, Jr.** 1940. Immunologic studies on patients with pneumococcic pneumonia treated with sulfapyridine. J. Clin. Invest. **19**:179–199.
6. **Grieco, M. H., J. D. Capra, and H. Paderon.** 1971. Reduced serum beta 1c/1a globulin levels in extrarenal disease. Am. J. Med. **51**:340–345.
7. **Guttman, I., and S. S. Wilks.** 1965. Introductory engineering statistics, p. 143–144. John Wiley & Sons, Inc., New York.
8. **Hinz, C. F., Jr.** 1956. Properdin levels in infectious and noninfectious disease. Ann. N.Y. Acad. Sci. **66**:268–272.
9. **Johnston, R. B., Jr., M. R. Klemperer, C. A. Alper, and F. S. Rosen.** 1969. The enhancement of bacterial phagocytosis by serum: the role of complement and two cofactors. J. Exp. Med. **129**:1275–1290.
10. **Johnston, R. B., S. L. Newman, and A. G. Struth.** 1973. An abnormality of the alternate pathway of complement activation in sickle cell disease. N. Engl. J. Med. **288**:803–808.
11. **Koethe, S. M., J. T. Casper, and G. E. Rodey.** 1976. Alternative complement pathway activity in sera from patients with sickle cell disease. Clin. Exp. Immunol. **23**:56–60.

12. **Mancini, G., A. O. Carbonara, and J. F. Heremans.** 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. Int. J. Immunochem. **2**:235–254.
13. **Mayer, M. M.** 1961. Complement and complement fixation, p. 135–139. *In* E. A. Kabat and M. M. Mayer (ed.), Experimental immunochemistry, 2nd ed. Charles C Thomas, Springfield, Ill.
14. **Pensky, J., C. F. Hinz, Jr., E. W. Todd, R. J. Wedgwood, J. T. Boyer, and I. H. Lepow.** 1968. Properties of highly purified human properdin. J. Immunol. **100**:142–158.
15. **Perrin, L. H., P. H. Lambert, V. E. Nyedegger, and P. A. Miescher.** 1973. Quantitation of C3PA (properdin factor B) and other complement components in diseases associated with a low C3 level. Clin. Immunol. Immunopathol. **2**:16–27.
16. **Reed, W. P., M. S. Davidson, and R. C. Williams, Jr.** 1976. Complement system in pneumococcal infections. Infect. Immun. **13**:1120–1125.
17. **Robertson, O. H., M. Hamburger, and L. A. Gregg.** 1953. On the nature of bacteremia in experimental pneumococcal pneumonia in the dog. I. Relationship of natural pneumococcidal-promoting activity of the serum to blood invasion. J. Exp. Med. **97**:283–296.
18. **Robinson, M. G., and R. J. Watson.** 1966. Pneumococcal meningitis in sickle-cell anemia. N. Engl. J. Med. **274**:1006–1008.
19. **Schutte, M., R. DiCamelli, P. Murphy, M. Sadove, and H. Gewurz.** 1974. C3 proactivator (C3PA) as an acute phase reactant. Clin. Exp. Immunol. **18**:251–256.
20. **Seeler, R. A., W. Metzger, and M. A. Mufson.** 1972. *Diplococcus pneumoniae* infections in children with sickle cell anemia. Am. J. Dis. Child. **123**:8–10.
21. **Tilghman, R. C., and M. Finland.** 1937. Clinical significance of bacteremia in pneumococcic pneumonia. Arch. Intern. Med. **59**:602–619.
22. **Van Metre, T. E., Jr.** 1954. Pneumococcal pneumonia treated with antibiotics. The prognostic significance of certain clinical findings. N. Engl. J. Med. **251**:1048–1052.
23. **West, C. D., N. C. Davis, J. Forristal, J. Herbst, and R. Spitzer.** 1966. Antigenic determinants of human beta 1C- and beta 1G-globulins. J. Immunol. **96**:650–658.
24. **Winklestein, J. A., J. A. Bocchini, Jr., and G. Schiffman.** 1976. The role of the capsular polysaccharide in the activation of the alternative pathway by the pneumococcus. J. Immunol. **116**:367–370.
25. **Winklestein, J. A., and R. H. Drachmann.** 1968. Deficiency of pneumococcal serum opsonizing activity in sickle cell disease. N. Engl. J. Med. **279**:459–466.
26. **Winkelstein, J. A., H. S. Shin, and W. B. Wood, Jr.** 1972. Heat-labile opsonins to pneumococcus. III. The participation of immunoglobulin and of the alternate pathway of C3 activation. J. Immunol. **108**:1681–1689.