INFECTION AND IMMUNITY, Sept. 1980, p. 1062-1066
0019-9567/80/09-1062/05$02.00/0

Vol. 29, No. 3

Alterations in Serum Opsonic Activity and Complement Levels in Pneumococcal Disease

G. SCOTT GIEBINK,[1]* THOMAS H. DEE,[2] YOUNGKI KIM,[1] AND PAUL G. QUIE[1]

Department of Pediatrics, University of Minnesota School of Medicine, Minneapolis, Minnesota 55455,[1] and the Section of Infectious Diseases, Department of Medicine, University of Wisconsin, Mt. Sinai Medical Center, Milwaukee, Wisconsin 53233[2]

Pneumococcal opsonic activity and concentrations of pneumococcal capsular polysaccharide antigen, C3, C4, factor B, C3, and factor B breakdown products were measured in the serum obtained acutely from 12 patients with serious pneumococcal disease. One patient showed markedly reduced pneumococcal opsonic activity, borderline-low C3, and the presence of C3 and factor B breakdown products and died. Although eight additional patients showed depressed levels of C3 or C4 or the presence of C3 or factor B breakdown products, none had reduced pneumococcal opsonic activity. All of the three remaining patients had normal opsonic activity and C3 and C4 levels. Covalescent serum was obtained from eight patients; six had normal C3 and C4 levels, and two had persistent C4 depression. These data show that complement is activated during pneumococcal disease and suggest that extensive complement activation may impair pneumococcal opsonic activity in certain patients and thereby compromise an important host defense mechanism.

The introduction of effective antimicrobial agents for the treatment of pneumococcal disease has reduced the mortality of these once life-threatening infections. Yet bacteremic pneumococcal pneumonia continues to be a frequent cause of death, especially during the first 24 h of hospitalization, and the prognosis for patients infected with certain pneumococcal serotypes has improved significantly less than other types (1). Pathogenesis of pneumococcal disease may be due in part to impaired killing of invading pneumococci. Lethality has been associated with the number of microorganisms in the blood (11) and reduction in serum complement levels (3, 4, 12-14). Depletion of C3 in mice by cobra venom treatment produced a marked increase in susceptibility to pneumococcal challenge (17). The means by which complement becomes activated is not known, although recent clinical (3, 14) and in vitro studies (6) have suggested an association between pneumococcal capsular polysaccharide (PCP) antigenemia and complement activation. To determine the role of complement in the pathogenesis of pneumococcal disease, pneumococcal opsonic function, PCP antigen, C3, C4, factor B, and C3 and factor B breakdown products were measured in the sera of 12 patients with serious pneumococcal infections.

(This paper was presented in part at the American Society for Clinical Investigation, San Francisco, 29 April to 1 May 1978.)

MATERIALS AND METHODS

The study population consisted of nine patients with pneumococcal pneumonia, four of whom were bacteremic, and three patients with bacteremic pneumococcal meningitis, one of whom also had endocarditis (Table 1). Two of the five nonbacteremic patients with pneumonia had *Streptococcus pneumoniae* isolated in pure culture from a transtracheal aspirate, and the remaining three patients had pneumococci isolated in pure culture from purulent sputum. All pneumococcal isolates were typed with group-specific antisera (Statens Serumsenstrate, Copenhagen, Denmark) and frozen at −70°C in buffered albumin. Six patients had underlying illnesses, including chronic alcoholism (patients 4 and 7), diabetes mellitus (patients 2 and 9), multiple myeloma (patient 1), and chronic obstructive pulmonary disease (patient 12). Six patients had no known underlying disease. Three patients expired during hospitalization. Death in each was attributed directly to pneumococcal disease and its complications.

Serial serum samples were obtained from these patients after informed consent was obtained and were stored at −70°C for the immunological studies. The initial acute serum was obtained within 48 h of admission. Subsequent sera were obtained every 24 to 72 h during hospitalization. In this report, the convalescent serum refers to the sample obtained after hospital day 4. Control sera were obtained from 15 healthy adult volunteers.

Serum opsonic activity was measured with a sensitive phagocytosis assay (5a, 7). Serum was tested for its ability to opsonize the patient's own pneumococcal isolate, which was mouse-passed immediately before the opsonic assay to ensure maximal capsulation. *S. pneumoniae* were grown in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) or in a synthetic medium lacking thymidine and adenine (5a) to which [*methyl*-^{3}H]thymidine was added. Live radiolabeled

pneumococci were incubated with 40% unheated and heat-activated (56°C for 30 min) patient and control sera for 60 min. The bacteria were washed and added to human polymorphonuclear leukocytes separated from the heparinized blood of healthy adult volunteers at a polymorphonuclear leukocyte:bacteria ratio of 1:10. The phagocytosis mixture was divided into aliquots and incubated with agitation for 30 min. After differential centrifugation, the percentage of pneumococci phagocytized was determined by the percentage of bacterial radioactivity in the neutrophil fraction. As such, serum opsonic activity was directly related to the percent phagocytosis of the test organism.

Serum PCP antigen was detected, typed and quantitated by counterimmunoelectrophoresis as described by Dee et al. (3). Minimum PCP antigen quantities detected for types 3, 4, 6, 9, 12, and 25 were 0.025, 0.5, 0.025, 0.1, 0.05, and 0.05 μg/ml, respectively. Assay for type 7 PCP was performed by Gerald Schiffman (State University of New York, Downstate Medical Center, Brooklyn, N.Y.) by using a type-specific radioimmunoassay with a sensitivity of 0.010 μg/ml, since it is poorly detected by usual counterimmunoelectrophoresis methodology due to its neutral charge.

Serum complement components C3, C4, and factor B were measured by the radial immunodiffusion technique of Mancini (Behring Diagnostics, Sommerville, N.J.) as described previously (3). Immunoelectrophoresis for C3 and breakdown products (C3c, C3d) was performed in 1.5% Ionagar with barbital buffer with 0.01 M ethylenediaminetetraacetic acid (pH 8.6). Immunoelectrophoresis was run for 2 h under constant voltage of 7 V/cm measured across the plate (10). Immunoelectrophoresis for factor B and breakdown products (Ba, Bb) was performed in 2% Noble agar with barbital buffer with 0.01 M ethylenediaminetetraacetic acid (pH 8.6) and run for 2 h under the same conditions.

RESULTS

Eight pneumococcal types accounted for the pneumococcal infections in the 12 patients studied (Table 1). Four patients had PCP antigen detected in their acute serum sample in concentrations from 0.2 to 32 μg/ml. Patient 4 with type 4 pneumococcal endocarditis and meningitis had the highest serum PCP concentration. Antigen was detected in the acute serum from three of seven bacteremic patients and in one of five nonbacteremic patients. All three patients who died were antigenemic on admission, whereas only one of nine survivors was antigenemic on admission ($P = 0.018$, Fisher exact test). Too few patients were studied to determine whether there was an association between particular serotypes and the presence of antigenemia.

Heat-labile and heat-stable opsonic activity in the patient's acute serum against that individual's infecting organism was compared with the opsonic activity of 15 control sera tested simultaneously against the patient's organism. Eleven patients showed heat-labile pneumococcal opsonic activity comparable to normal, and all showed negligible heat-stable opsonic activity (Fig. 1a and b). There was insufficient serum from patient 6 to test heat-stable activity. Patient 3, who had type 12 pneumococcal bacteremia and meningitis, 0.8 μg of capsular antigenemia per ml, borderline-depressed serum C3, and the presence of C3c, C3d, Ba, and Bb fragments, showed markedly depressed heat-labile pneumococcal opsonic activity and died.

TABLE 1. *Pneumococcal types and acute serum concentrations of antigen, C3, C4, factor B, C3, and factor B breakdown products in patients with pneumococcal disease*

Focus of pneumococcal infection	Patient no.	Pneumococcal type	Outcome	PCP antigen (μg/ml)	C3 (55–120 mg/dl)[a]	C4 (20–50 mg/dl)[a]	Factor B (10–45 mg/dl)[a]	Breakdown products			
								C3c	C3d	Ba	Bb
Bacteremic meningitis	1	6	Survived	ND[b]	114	9[c]	30	ND	+	ND	ND
	2	3	Died	0.2	112	68	31	ND	+	ND	ND
	3	12	Died	0.8	56	39	22	+	+	+	+
Bacteremic pneumonia	4	4	Died	32.0	44[c]	26	15	ND	ND	ND	+
	5	4	Survived	ND	128	23	44	ND	ND	ND	ND
	6	7	Survived	ND	64	43	17	NT[d]	NT	NT	NT
	7	25	Survived	ND	98	36	32	ND	+	ND	ND
Nonbactermic pneumonia	8	3	Survived	ND	96	38	28	ND	+	ND	ND
	9	3	Survived	ND	98	66	38	ND	+	ND	ND
	10	9	Survived	ND	58	29	19	ND	+	ND	ND
	11	9	Survived	9.0	88	40	42	NT	NT	NT	NT
	12	33	Survived	ND	64	15[c]	13	ND	ND	ND	ND

[a] Normal range.
[b] ND, none detected
[c] Below normal range
[d] NT, Not tested

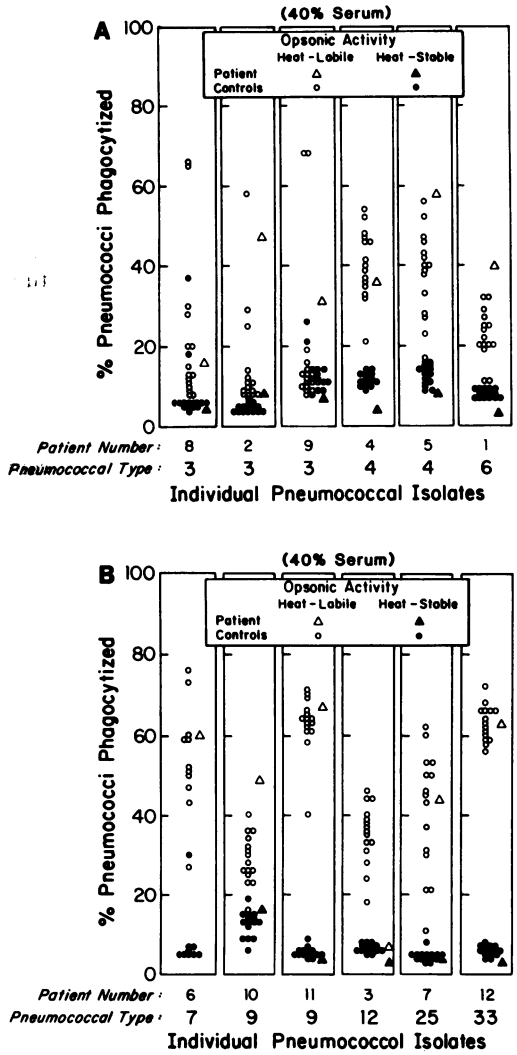

FIG. 1. *Heat-stable and heat-labile serum opsonic activity in the acute serum from 12 patients with pneumococcal disease tested with 40% serum and the patient's pneumococcal isolate. Results are compared with 15 normal control sera tested simultaneously.*

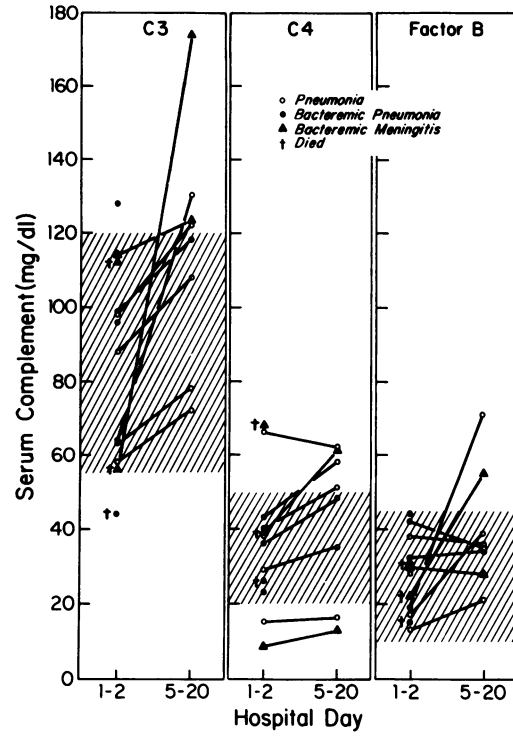

FIG. 2. *Serum C3, C4, and factor B concentrations in 12 patients with pneumococcal disease including nonbacteremic pneumonia, bacteremic pneumonia, and bacteremic meningitis. Acute serum was obtained during the first 48 h of hospitalization, and convalescent serum was obtained after hospital day 4. Three patients died. The shaded area indicates the normal range.*

Serum complement components, C3, C4, and factor B were measured in the acute and convalescent sera from these patients to determine whether opsonic deficiency might be related to complement depletion. Three patients had reduced concentrations of C3 or C4 in serum, and two additional patients (no. 3 and 6) had borderline-low C3 levels. Convalescent sera obtained from eight of the nine survivors showed increasing C3, C4, and factor B concentrations in most patients, although two patients had persistently depressed C4 levels (Fig. 2). Complement breakdown products were measured to detect evidence for persistent complement activation. Of the 10 sera tested for complement breakdown products, 10 showed C3d fragments, and one of these (patient 3) showed C3c fragment. Two sera showed Bb fragment, and one of these (patient 3) showed Ba fragment (Table 1). Although patient 3 died, serum obtained on hospital day 9 showed normal C3 (174 mg/dl) and presence of C3d fragment without C3c, Ba, or Bb fragments.

DISCUSSION

Despite the use of highly effective bactericidal drugs, the mortality of bacteremic pneumococcal pneumonia remains high (20%) and is even higher in patients with extrapulmonary foci of infection (1). Mortality increases significantly after the sixth decade of life and in patients with complicating illness. In the present study all three patients who died were bacteremic, and two had extrapulmonary foci of infection.

Patients with lethal pneumococcal disease have higher levels of serum PCP antigen, as was found in our patients (3). The quantity of antigen

may reflect an increased number of organisms in the blood of these individuals, since there is a direct relationship between fatality and number of circulating pneumococci in experimental animals (11). Patients who die also have lower levels of antipneumococcal capsular antibody and lower serum complement levels (3). Activation of the alternative complement pathway has been found in both bacteremic and nonbacteremic patients, although activation of complement has been greater in bacteremic individuals (2).

Efficient killing of pneumococci requires the participation of serum opsonic factors, including complement and immunoglobulin, and of polymorphonuclear leukocytes. Studies in rodent sera have shown that the interaction of pneumococci with complement results in activation of C3 and fixation of C3b, the opsonically active fragment, on the surface of the pneumococcus (9, 15). Although immunoglobulin participates in the interaction of pneumococci and complement, it is not known whether immunoglobulin is required for this interaction (16). Most pneumococcal serotypes activate C3 via the alternative pathway (5), are efficiently opsonized in human serum in the absence of a functional classical pathway, and can be moderately opsonized in the absence of immunoglobulin G (7). Experimental complement depletion in mice has been shown to reduce serum opsonic activity and increase mortality (17). Serum opsonic activity against the infecting pneumococcal serotypes was assayed in this study to determine the biological consequences of complement activation in patients with pneumococcal bacteremia.

Serum obtained acutely from 9 of the 12 patients showed evidence of complement activation, including depressed levels of C3 and C4 or the presence of C3 or factor B breakdown products, but only one patient showed reduced pneumococcal opsonic activity. The frequency of reduced pneumococcal opsonic activity may have been underestimated, since 40% serum was employed in the opsonic assay and lower serum concentrations might have magnified subtle opsonic deficiencies. Although evidence of complement activation was found in most patients, C3c and Ba fragments were detected only in the single patient with reduced pneumococcal opsonic activity who died. Because the measurement of C3c is less sensitive than C3d, this patient may have had greater complement activation than the others. C3 and factor B breakdown products were present irrespective of the absolute serum levels of C3 or factor B. Since serum was used in this study, one cannot absolutely exclude the possibility of accelerated in vitro breakdown of factor B or C3 by storing, freezing, and thawing. However, in our experience, normal sera stored at −70°C for as long as 3.5 years, and frozen and thawed up to five times, do not show any detectable breakdown products of these complement components (10).

Evidence of complement activation was found as often in antigenemic and nonantigenemic patients, suggesting that complement activation may have been due to other subcellular components of the pneumococcus. Pneumococcal cell wall, and in particular cell wall teichoic acid, has been shown to activate complement more readily that PCP (18). We have found that C carbohydrate extracted from type 7 pneumococci also activated complement via the alternative pathway but less efficiently than does type 7 PCP (G. S. Giebink, unpublished data). Circulating PCP antigen-antibody complexes have been found in patients with both complicated and uncomplicated forms of pneumococcal disease (T. H. Dee, G. Schiffman, C. K. Dham, J. Boudrealt, and M. W. Rytel, unpublished data). Reports of acute glomerulonephritis with hypocomplementemia and immune complex deposition in the glomeruli of these patients suggest a pathological role for the immune complexes (9, 14).

These data suggest that reduced pneumococcal opsonic activity may be important in the pathogenesis of serious pneumococcal disease in certain patients. Prolonged complement activation may result in depletion of the opsonic system and fresh plasma might help correct this host defense deficiency.

ACKNOWLEDGMENTS

This study was supported in part by Public Health Service grants AI-08821, AI-42521, and AI-00455 and contract NOI-AI-52533 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Austrian, R., and J. Gold.** 1964. Pneumococcal bacteremia with especial reference to bacteremic pneumococcal pneumonia. Ann. Int. Med. **60**:759–776.
2. **Coonrod, J. D., and B. Rylko-Bauer.** 1977. Complement levels in pneumococcal pneumonia. Infect. Immun. **18**:14–22.
3. **Dee, T. H., G. Schiffman, M. I. Sottile, and M. W. Rytel.** 1977. Immunologic studies in pneumococcal disease. J. Lab. Clin. Med. **89**:1198–1207.
4. **Dick, F. G.** 1912. On the development of proteolytic ferments in the blood during pneumonia. J. Infect. Dis. **10**:383–387.
5. **Fine, D. P.** 1975. Pneumococcal type-associated variability in alternate complement pathway activation. Infect. Immun. **12**:772–778.
5a. **Giebink, G. S., J. E. Foker, Y. Kim, and G. Schiffman.** 1980. Serum antibody and opsonic responses to pneumococcal capsular polysaccharide vaccination in normal and splenectomized children. J. Infect. Dis. **141**: 404–412.
6. **Giebink, G. S., J. V. Grebner, Y. Kim, and P. G. Quie.**

1978. Serum opsonic deficiency produced by *Streptococcus pneumoniae* and by capsular polysaccharide antigens. Yale J. Biol. Med. **51**:527–538.

7. **Giebink, G. S., J. Verhoef, P. K. Peterson, and P. G. Quie.** 1977. Opsonic requirements for phagocytosis of Streptococcus pneumoniae types VI, XVIII, XXIII, and XXV. Infect. Immun. **18**:291–297.
8. **Hyman, L. R., E. H. Jenis, G. S. Hill, S. W. Zimmerman, and P. M. Burkholder.** 1975. Alternative C3 pathway activation in pneumococcal glomerulonephritis. Am. J. Med. **58**:810–814.
9. **Johnson, 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 components and two co-factors. J. Exp. Med. **129**:1275–1290.
10. **Kim, Y., K. Miller, and A. F. Michael.** 1977. Breakdown products of C3 and factor B in hemolytic-uremic syndrome. J. Lab. Clin. Med. **89**:845–850.
11. **Perry, J. E., and L. E. Cluff.** 1966. Clinical and bacteriological events in experimental lethal pneumococcal infection. Tex. Rep. Biol. Med. **24**:125–140.
12. **Reed, W. P., M. S. Davidson, and R. C. Williams, Jr.** 1976. Complement system in pneumococcal infections. Infect. Immun. **13**:1120–1125.
13. **Rutstein, D. D., and W. H. Walker.** 1942. Complement activity in pneumonia. J. Clin. Invest. **21**:347–352.
14. **Rytel, M. W., T. D. Dee, J. E. Ferstenfeld, and G. T. Hensley.** 1974. Possible pathogenetic role of capsular antigens in fulminant pneumococcal disease with disseminated intravascular coagulation (DIC). Am. J. Med. **57**:889–896.
15. **Shin, H. S., M. R. Smith, and W. B. Wood, Jr.** 1969. Heat labile opsonins to pneumococcus. II. Involvement of C3 and C5. J. Exp. Med. **130**:1229–1239.
16. **Winkelstein, J. A., and H. S. Shin.** 1974. The role of immunoglobulin in the interaction of pneumococci and the properdin pathway: evidence for its specificity and lack of requirement for the Fc portion of the molecule. J. Immunol. **112**:1635–1642.
17. **Winkelstein, J. A., M. R. Smith, and H. S. Shin.** 1975. The role of C3 as an opsonin in the early stages of infection. Proc. Soc. Exp. Biol. Med. **149**:397–401.
18. **Winkelstein, J. A., and A. Tomasz.** 1978. Activation of the alternative complement pathway by pneumococcal cell wall teichoic acid. J. Immunol. **120**:174–178.