Consumptive Opsoninopathy:

Possible Pathogenesis in Lethal and Opportunistic Infections

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Serum levels of properdin, Factor B and C3 and the ability of these sera to opsonize *E. coli* 075 were measured in 17 patients with surgical infections ranging in severity from mild to fatal. There was good direct correlation between severity of infection, serum levels of properdin and C3, and the ability of the serum to support opsonization. The levels of Factor B were not significantly reduced when measured by radial immunodiffusion, but immunoelectrophoresis showed conversion. Restoration of full opsonic activity was accomplished only by the addition of a combination of C3, Factor B, and properdin in excess. The findings provide evidence that severe bacterial infection causes a consumption of opsonic proteins which may result in a reduced ability of the patient's serum to opsonize bacteria and thereby further increase susceptibility to infection.

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IMMUNOLOGICAL defense mechanisms have been known to participate in recovery from infection for three quarters of a century. However, only recently has there been even partial elucidation of the complex biochemical mechanisms involving the alternative pathway of complement which participates in such an important way to resistance to infection by many bacteria. The present investigation provides evidence that components of the alternative pathway may be markedly diminished in the sera of patients during severe infection, associated with a decreased ability of those sera to support opsonization of bacteria. The findings also suggest that severe infection can result in a consumptive condition of serum opsonins which further increases susceptibility to infections by microbial pathogens. This condition is felt to contribute both to the development of superinfections and irreversibility of established infection.

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In this paper, the term alternative pathway is used for alternate pathway or properdin pathway; Factor B is used for C3 proactivator and Factor D is used for C3 proactivator convertase.

Methods

Seventeen surgical patients with infections of varying degrees were studied at or near the time of infection. A brief clinical summary for each patient is given in Table 1.

Collection and Preparation of Serum Samples

Ten to 15 milliliters of whole blood was drawn from each patient and allowed to clot at room temperature for two hours. The serum was removed by centrifugation, frozen, and stored at −70°. Before immunochemical or functional tests were done, antibiotics were removed from each sample by dialysis for 24 hours against Hank's balanced salt solution (HBSS) at 4° to prevent decomposition of heat labile complement components. The samples were then divided into one to two ml aliquots and those not tested immediately were frozen (−70°). Previous tests showed that opsonic activity of normal serum was not changed by freezing or dialysis at 4° for 24 hours.

TABLE 1. *Abbreviated History of Patients in Study*

Patients With Fatal Infections

Patient 1: 58-year-old man received cadaveric renal transplant. Died from gram-negative septicemia complicating pulmonary nocardiosis 14 months later. Blood sample on day of death, less than 24 hours after septicemia developed.

Patient 2: 46-year-old man received cadaveric renal transplant 8/27/73. Developed severe herpes zoster with slow recovery. Perforated diverticulum of colon on 1/17/74 and died from peritonitis on 1/19/74. Blood for studies drawn on day of death. Patient was hypotensive for 48 hours before death.

Patient 3: 41-year-old man received cadaveric kidney transplant 4/2/74. Became septic on 5/2/74. Transplant nephrectomy on 5/9/74. Continued to be septic. Mildly hypotensive 5/26 after first sample drawn. Studied on 5/26, 5/27, and 5/28. Shock on 5/29/74; died same day. Cause of death was subphrenic abscess, Pseudomonas pneumonia and septicemia.

Patient 4: 12-year-old boy with 91% total body burn, 85% 3°. Blood samples PBD 14 and 19 at time of septicemia. Died PBD 27 from gram negative sepsis, gangrene of left arm, and perforated duodenal ulcer.

Patient 5: 78-year-old man with obstructive uropathy, diabetes mellitus and vascular disease. Admitted and operated on 5/11/74 because of perforation of cecum and peritonitis. Studied on 5/12 and 5/14. Leg amputated on 5/17 for arteriosclerotic gangrene. Leakage from cecostomy on 5/20. Progressive peritonitis and death on 5/23/74.

Patients With Nonfatal Life Threatening Infections

Patient 6: 2-year-old boy. Burned on 7/9/73: 82% total body burn, 76% 3°. Transferred to unit on 7/13. Positive blood cultures on 7/24, 7/25, 7/31, and 8/4. Studied on 8/6. Recovered and discharged on 2/8/74.

Patient 7: 80-year-old woman with colon resection for carcinoma on 4/17/73. Leaking anastomosis and peritonitis on 4/28, treated by diversion and antibiotics. Tested for opsonic activity on 5/1. Gradually recovered from the peritonitis with antibiotic therapy, but developed a pulmonary superinfection from *Pseudomonas aeruginosa* on 5/16.

Patient 8: 38-year-old woman. Cadaveric renal transplant on 10/22/74. Poor function. Developed hemophilus pneumonia, herpes and possible lung abscess on 11/9/74. Studied on 11/13. Gradual recovery after immunosuppressive therapy stopped. Nephrectomy on 11/27.

Patient 9: 66-year-old woman. Admitted on 10/17/73 with hip fracture. Open reduction and nailing on 10/31. Wound infection with *C perfringens* on 11/14. Studied on 11/20. Infection failed to clear and hip disarticulated on 4/3. Discharged on 6/12.

Patient 10: 18-year-old man admitted on 1/18/74 with gunshot wound to liver, aorta, small intestine. Developed renal failure and Pseudomonas pneumonia postoperatively. Studied on 1/30. Recovered from pneumonia, but later died on 3/11 from sepsis caused by *Candida albicans* and *P. mirabilis*.

Patients With Acute Brief Infections, Recurrent Infections or Infections Resulting From Anatomical Cause

Patient 11: 48-year-old man. Cadaveric renal transplant on 11/24. Acute pneumonia and bacteremia on 11/9 with Klebsiella. Prompt clinical response with antibiotics. Afebrile on 11/11/74. Studied on 11/13.

Patient 12: 23-year-old woman, nurse. Recurrent self inflicted infections. Studied 2 days after she injected herself in hip with hand lotion, causing deep abscess.

Patient 13: 36-year-old man with early chronic renal failure and extensive hidradenitis suppritivum of perineum, groin and axilla. Studied before multiple excisions started. No systemic infection. Now healed.

Patient 14: 43-year-old man. Renal transplant on 7/8/71. Developed osseous involvement and meningitis from Aspergillus. Treated with amphotericin. Studied 1/24/73. Died on 1/27/74 from myocardial infarction.

Patient 15: 23-year-old woman with juvenile diabetes mellitus with recurrent infections. Admitted on 7/2 for abscess in groin and axilla. Multiple I & D's. Tested on 7/6 and 7/10. Recovered and discharged on 7/16. Admitted several times since with various minor infections.

Patient 16: 62-year-old man with recurrent staphylococcal infections of herniorraphy wound. Studied in quiescent phase.

Patient 17: 25-year-old woman with systemic lupus erythematosus. Recurrent pyelonephritis. Subfascial abscess leg 5/17/74. Subphrenic abscess on 9/17/74. *E. coli* septicemia on 10/11/74. Continued spiking fevers. Blood sample for study on 10/31/74. *E. coli* septicemia again on 11/29 and 12/17 to 12/23/74. Cholecystectomy for stones and drainage of abscess on 1/3/75. Discharged on 3/8/75. Cause of recurrent septicemia was gall bladder disease and stones.

Pooled normal human serum (PNHS) was prepared from similarly treated blood samples taken from five normal donors. The sera were pooled, thoroughly mixed, divided into 5 ml aliquots and stored frozen at −70°. This single lot of PNHS served as a reference for both the opsonization studies and immunochemical determinations.

Immunochemical Determination of Concentrations of C3, Properdin, and Factor B

Concentrations of these components of the alternative pathway were measured by standard radial immunodiffusion. C3 concentration was measured both against β1c/β1a (Behring Diagnostics) and against the B antigen of C3 to detect only functionally intact C3. Antisera against the C3 (B) antigen was prepared in our laboratory according to the method of Molengar et al.[19] Antibody to Factor B was purchased from Behring Diagnostics. Properdin was prepared by the method of Pensky et al.,[23] and goats were immunized to prepare specific anti-properdin antibody. The method of Westberg et al.[29] was used to further clarify precipitin rings. Since the concentrations of C3, properdin and Factor B were related to tests for opsonic activity, values were expressed as a percentage of the PNHS reference serum, taken to be 100%. Immunoelectrophoresis was performed in 1% agarose, using barbital buffer, pH 8.6.

Opsonic Activity of Serum

Tests for opsonic support of serum were performed by a modification of a previously described technique[3] in Falcon polypropylene tubes, each containing 5

TABLE 2. *Alternative Pathway Profile for Patients With Infections of Differing Severity*

Severity of Infection	C3 (B Determinant % of Normal	C3 (β1c/β1a) % of Normal	Properdin % of Normal	Factor B % of Normal
Fatal infections				
Patient 1	4	ND	ND	123
Patient 2	0	20	25	80
Patient 3	16	18	26	77
Patient 4	40	45	60	100
Patient 5	34	26	85	90
(Average)	(19)	(27)	(49)	(94)
Nonfatal life threatening infections				
Patient 6	32	ND	ND	86
Patient 7	22	25	26	88
Patient 8	60	30	75	100
Patient 9	56	68	ND	100
Patient 10	33	ND	65	115
(Average)	(41)	(41)	(55)	(98)
Acute brief infections, recurrent infections, or infections resulting from anatomical cause				
Patient 11	48	ND	100	100
Patient 12	167	85	ND	134
Patient 13	40	101	88	175
Patient 14	175	ND	ND	137
Patient 15	108	58	55	83
Patient 16*	43	32	73	77
Patient 17	70	80	100	175
(Average)	(93)	(71)	(83)	(126)

* This patient was studied 6 months later and all values were normal.
ND = not done.

× 10^6 normal human neutrophils, a final concentration of 5% patient serum of PNHS (except where indicated), 1 × 10^6 *E. coli* 075 and tissue culture medium consisting of HBSS with 10 unit of heparin and 1 mg of gelatin per ml. Neutrophils were obtained from normal healthy volunteers. The total volume of the reaction mixture was 1.0 ml. HBSS was substituted for the neutrophils in control tubes. The tubes were then incubated at 37° for three hours on an Ames tilting table aliquot mixer. At the end of the incubation period, samples were removed from the tubes, and dilutions made on trypticase soy agar to determine the number of bacteria surviving opsonophagocytosis. The plates were counted after 24 hours incubation at 37° and calculations were made to assess the per cent kill.

E. coli 075 is known to require participation of the alternative pathway of complement for opsonization and killing by human neutrophils.[14] This organism was generously provided by Dr. Hugo Jasin of the University of Texas Southwestern Medical School.

In one patient, purified properdin, Factor B and C3 were added singly or in combination in an attempt to restore opsonic activity. The Factor B was provided by Dr. Otto Gotze (Scripps Clinic and Research Foundation, La Jolla, California), the C3 was purchased from Cordis, and the functionally active properdin was prepared in our laboratory by affinity chromatography.[21]

Results

The profile for each patient studied for selected components known to participate in opsonic activity of the alternative pathway is shown in Table 2. When these patients were catagorized according to the severity of their infection, a good correlation could be demonstrated between severity of infection and the profile of alternative pathway complement components. In addition, a statistically significant correlation was demonstrable between the serum levels of C3 measured by the B determinant and the ability of the serum to support opsonization of *E. coli* (Fig. 1). Measurement of C3 by the standard β1c/β1a antisera did not appear to be as accurate since it also detected inactive fragments after conversion.

Levels of Factor B were not significantly reduced when measured by immunodiffusion, but immunoelectrophoresis of the sera showed that there was a change in mobility from the β to γ position, indicating that the molecule had been converted to the inactive form (Fig. 2). In addition, repletion with functionally intact Factor B was necessary to restore opsonic activity to the markedly defective serum of patient 3. However, addition of purified components significantly restored opsonic activity only when C3, Factor B and properdin

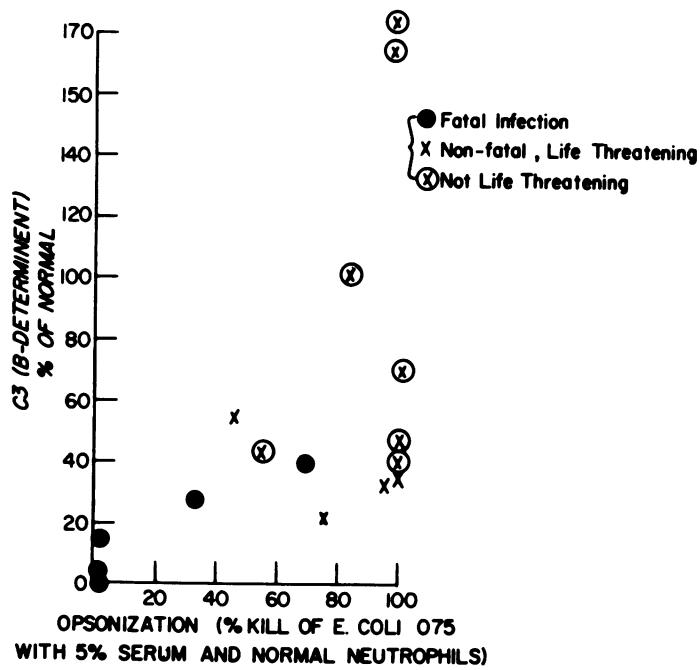

FIG. 1. Comparison of serum levels of C3 with ability of each patient's serum to support opsonization of *E. coli* 075 for phagocytosis and killing by normal human neutrophils. The correlation is significant ($P < 0.05$).

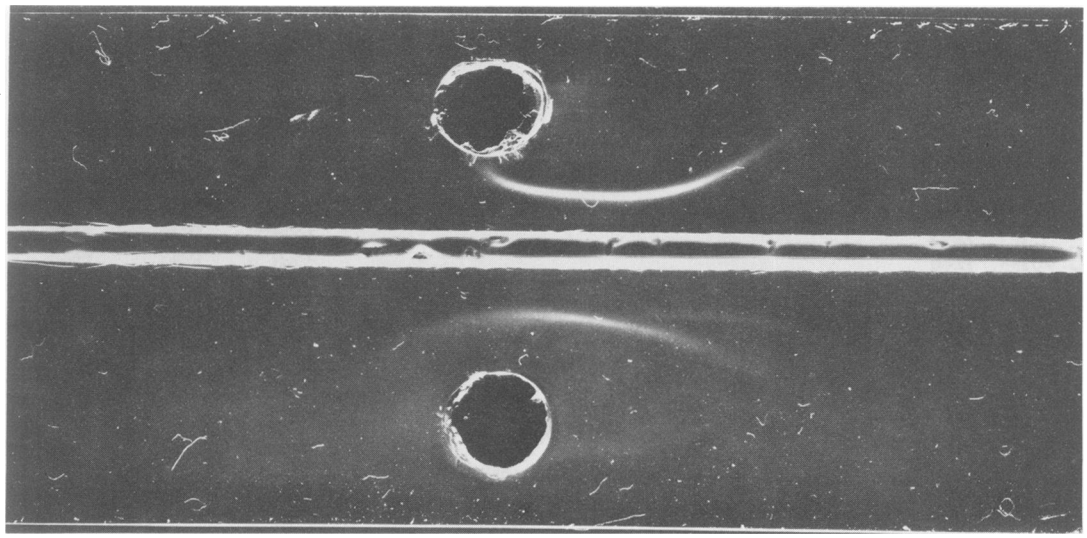

FIG. 2. Immunoelectrophoresis of PNHS (upper well) and serum from patient 3 (lower well). Anode is on right. Center trough contains antisera specific for Factor B. A shift in migration toward the cathode indicates conversion of the majority of the patient's Factor B.

were added in combination (Figs. 3 and 4). These results indicate that measurement of Factor B by radial immunodiffusion detects both active and inactive forms and that normal values so obtained do not necessarily reflect functional integrity.

Opsonization of *E. coli* 075 in the presence of 5% NPHS consistently resulted in 99.0% to 99.9% kill in the presence of normal neutrophils during the three hour period of incubation. Dilution of PNHS resulted in good opsonic support at 3% concentration, but there was a very sharp reduction in opsonic support with 1% or 2% PNHS (Fig. 5). This sharp reduction provided a means to estimate the extent of depletion in the limiting factor(s) for opsonization in patient's serum. Reductions of the bacterial count in these opsonization tests of less than 95% were taken to be indicative of significant loss of opsonic activity using 5% serum. In the example shown in Fig. 5, the patient's serum supported opsonization poorly at 4% but relatively well at 5%. In this patient, therefore, it was estimated that the limiting factor(s) in the patient's serum was reduced by approximately 40% compared to NPHS. Thus, in Figure 1, values for per cent kill less than 95% probably represented a reduction of 50% or more of limiting opsonic protein(s).

Discussion

At the turn of the century, Wright and Douglas[31] demonstrated that serum factors had a facilitating effect

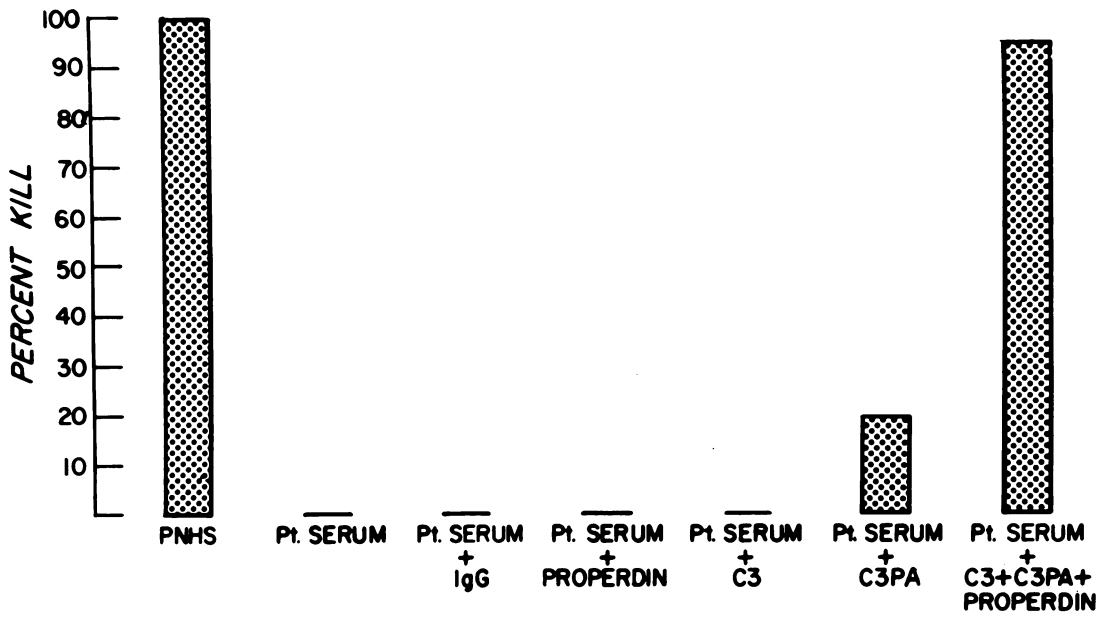

FIG. 3. Percentage of an inoculum of *E. coli* 075 killed by normal neutrophils when incubated in the presence of 4% PNHS or 4% serum from patient 3 (patient serum). The following amounts of purified components were added to the patient's serum as indicated: IgG 16 mgm; C3 128 μg; Factor B 32 μg; and properdin 1.6 μg. Only the combined addition of C3, Factor B and properdin (in excess) significantly restored opsonic activity. C3PA = Factor B.

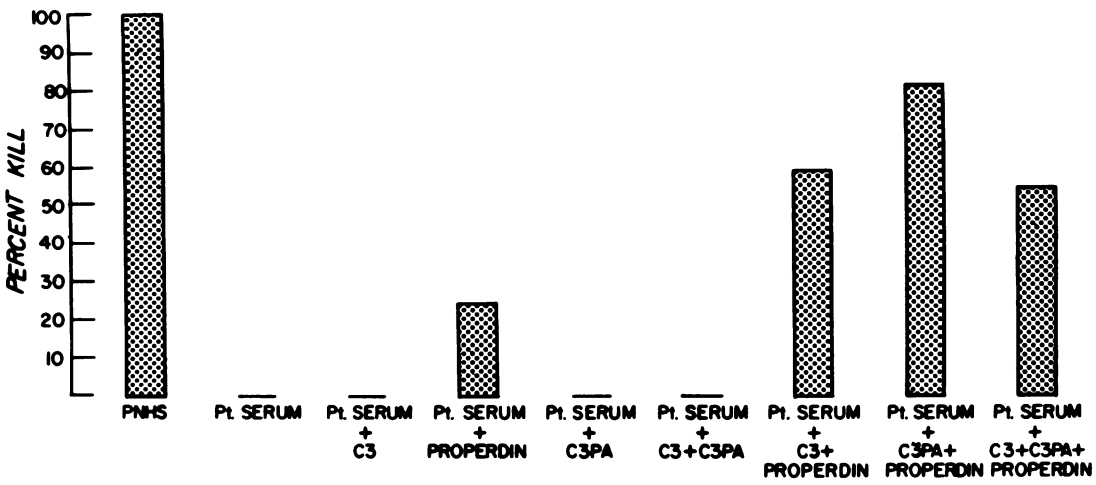

FIG. 4. Percentage of an inoculum of *E. coli* 075 killed by normal neutrophils when incubated in the presence of 3% PNHS or 3% serum from patient 3 (patient serum). The following amounts of purified components were added to the patient's serum where indicated: C3 24 μg; Factor B 5.4 μg; properdin 0.6 μg. In contrast to the preceding figure, properdin gave very slight restoration but was obviously not the only limiting factor. The data suggest that Factor B and properdin are more limiting than C3 and that other proteins are involved. C3PA = Factor B.

on phagocytosis and killing of pathogenic bacteria. These factors were later found to be related to heat stable antibody and heat labile complement components.

In 1933, Ward and Enders[28] showed that the heat labile activity was related to complement. The classical complement pathway is now reasonably understood to involve cascading interactions from C1 to C9, usually being initiated by specific antibodies of the IgG or IgM class. Within the last decade, it has been recognized that the distal components of complement can be activated through a different mechanism(s) now known variously as the alternative pathway, alternate pathway, bypass mechanism, C3 shunt, or properdin pathway (see Osler et al.[22] or Muller-Eberhard[20] for reviews). The present concept of alternative pathway activation involves several proteins including properdin, C3, Factor B, Factor D, and others still less well defined.

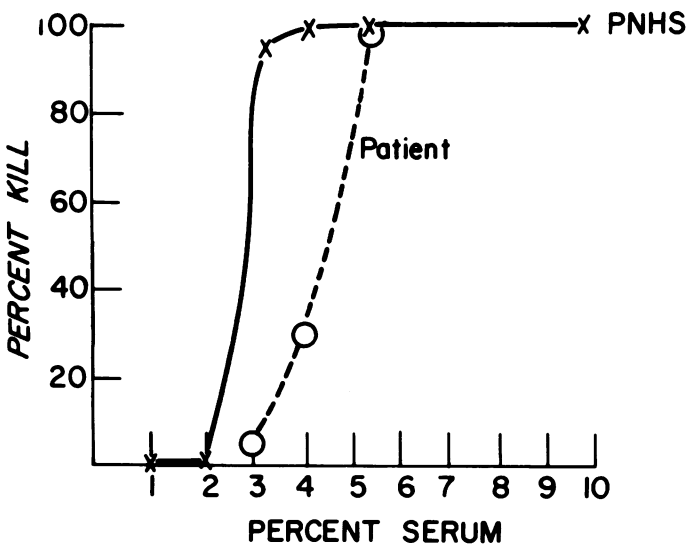

FIG. 5. Percentage of an inoculum of *E. coli* 075 killed by normal neutrophils when incubated in the presence of varying concentrations of PNHS or patient serum (patient 5). An abrupt drop in opsonic activity occurred between 3% and 2% PNHS compared to between 5% and 4% for the patient's serum. The limiting factor(s) for opsonization should not decrease opsonization in 5% serum concentration until reduced in activity by approximately 50%. The C3 for patient 5 was 34% of PNHS and the properdin 85%.

C3 has been recognized as the most important of the opsonic proteins in the complement pathway, and many diseases with biochemically low levels of C3 have been associated with infection, including inherited homozygous deficiency of C3, reported by Alper et al.,[5] hypercatobolism of C3 reported by Alper et al.,[4] and systemic lupus erythematosus, reported by Hunsicker et al.[13] Studies by McCracken et al.[18] and Forman et al.[10] of premature infants with low birth weight have shown that C3 levels were significantly lower in smaller sized babies, with a relationship between the C3 levels and opsonic activity of the serum from children. The pivotal role of C3 activated via the alternative pathway in resistance to bacterial infection has also been suggested by the relatively good health of humans and animals with genetic defects in C2, C4, and C6.[15,22,24] Johnson et al.[15] suggested that opsonic activity of human serum deficient in C2 was effected through the alternative pathway since treatment with inulin or with cobra venom factor, both of which destroy native C3 by activation of the alternative pathway, resulted in loss of opsonic activity. Serum deficient in C2 had normal opsonic activity before such treatment. Root et al.[24] showed that guinea pig serum genetically deficient in C4 had a slower rate of killing compared to normal sera, and that this activity could be restored by the addition of small amounts of purified guinea pig C4, suggesting that the alternative pathway of complement was essen-

tial for opsonization and killing by sera, but C4 was necessary for maximum activity. Their observations are consistent with those of Spitzer et al.[26] who reported that one of the fragments generated during the activation of properdin activated C4. Jasin[14] showed participation of the alternative pathway of complement was definitively needed for opsonization of *E. coli* 075. In his studies, opsonization occurred normally in human serum lacking in Clq or immunoglobulins. Treatment of serum at 50° for 30 minutes to destroy Factor B markedly reduced opsonic activity, but this activity could be restored by the addition of purified Factor B. Stossel et al.[27] demonstrated that paraffin oil containing oil red 0 emulsified with lipopolysaccharide from *E. coli* was ingested by guinea pig neutrophils after opsonization by fresh homologous serum which was associated with fixation of C3 to the lipopolysaccharide particles. Serum genetically deficient in C3 had no opsonic activity, but this could be restored by the addition of C3. Normal and C4 deficient guinea pig serum and normal and C2 and C4 deficient human serum were all equally effective in opsonizing the particles. Serum deficient in Factor B had diminished activity which was restored by the addition of Factor B. Diamond et al.[8] showed that serum depleted in properdin or Factor B was markedly deficient in opsonic activity against *Cryptococcus neoformans*. However, the classical complement pathway was also found to participate in opsonization against this organism, and Mergenhagen et al.[16] also showed that endotoxin could initiate lysis of erythrocytes via the classical pathway. Their study suggested that at least for some bacteria, IgG may be an initiator of the alternative pathway. Winkelstein and Shin[30] have also shown that IgG2 can participate in the interaction of pneumococci and *E. coli* in the alternative pathway, but their studies did not demonstrate that immunoglobulin was required.

The consumption of antibody during acute infections has been known for some time. Recently, we demonstrated that selective consumption of type specific anti-pseudomonas antibody occurred during infections with *Pseudomonas aeruginosa* in burns,[1] and the deteriorating clinical courses could be reversed by the passive administration of specific hyperimmune antibody.[2] Changes in complement level have also been noted during infections in experimental animals. Gilbert and Braude[11] demonstrated that both antibody to endotoxin and hemolytic complement levels fell rapidly and usually together after the injection of lethal amounts of endotoxin into rabbits. Spink et al.[25] showed decreases in hemolytic complement titers in dogs after endotoxin administration. Activation of the alternative pathway during infection was described soon after the discovery of properdin. Hinz[12] reported in 1956 that low levels of properdin occurred in humans with gram-negative infections and bacterial pneumonia. More recently, McCabe[17] demonstrated that serum levels of C3 were significantly reduced in patients with shock secondary to bacteremia caused by gram-negative organisms, but uncomplicated bacteremia was not associated with diminished C3 levels. In an expansion of this work, Fearon et al.[9] showed that the alternative pathway of complement was involved whereas the classical pathway was not. They interpreted their findings to suggest that the products released during activation of the alternative pathway contributed to development of the shock. This supposition is supported by earlier experimental data in animals,[11,25] and by the work of Bokisch et al.[7] who studied levels of complement components in patients with dengue hemorrhagic shock syndrome. All of the complement components except C9 decreased during shock and seemed to be related to severity of the disease. However, Bach et al.[6] described 7 patients in whom gram negative bacteremia developed following transurethral resection. In two of these, β1c (C3) dropped remarkably, but only one of these developed shock. Of the five patients in our study with lethal infection, three developed low levels of C3 before the onset of hypotension.

Our findings, taken together with the data just discussed, provide strong evidence that many bacterial infections in man can result in a consumption of opsonic proteins which are critical for antibacterial defense. There has been excellent correlation between the clinical course of the patients studied thus far and the opsonic activity of their serum. It would appear that if the synthetic rate of these proteins is not sufficiently great to counteract the consumptive process, serum levels and the ability to opsonize bacteria will fall. We have termed this process a consumptive opsoninopathy[1] and postulate that if the consumptive condition results in sufficiently low levels of the important serum opsonins, a condition will develop in which the patient's ability to opsonize bacteria is severely compromised. A vicious cycle may be produced which can cause both increased susceptibility to superinfection or progression of the infection with release of anaphylotoxins, septic shock, and death. Progressive lack of resistance associated with overwhelming infection could easily explain the occurrence of positive blood cultures with multiple organisms so commonly seen in patients in extremis from infection.

These studies suggest that replenishment of opsonic proteins to restore normal opsonic activity may benefit patients with severe infections, and a well controlled clinical trial of the administration of opsonic proteins to patients with life-threatening infections may be warranted after appropriate baseline studies. We have re-

cently demonstrated that whole blood stored for up to 21 days contains all of the components necessary for opsonization with only slight reduction of activity compared to fresh blood, and plasma from which the cryoprecipitate has been removed also retains full opsonic capability. However, preliminary studies need to be performed to determine the amount of replacement therapy which might be necessary to correct deficiencies of the magnitude described in this study and to determine that septic shock will not be aggravated via release of vasoactive mediators during replacement therapy.

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