INCREASED SUSCEPTIBILITY TO INFECTION ASSOCIATED WITH 
ABNORMALITIES OF COMPLEMENT-MEDIATED FUNCTIONS AND OF THE THIRD 
COMPONENT OF COMPLEMENT (C3)*

CHESTER A. ALPER, M.D., NEIL ABRAMSON, M.D., RICHARD B. JOHNSTON, JR., M.D., 
JAMES H. JANDL, M.D., AND FRED S. ROSEN, M.D.

Abstract In a patient with Klinefelter's syndrome and lifelong increased susceptibility to infection, no abnormalities were found in humoral antibody production, cellular immunity or leukocyte function. In contrast, the patient's serum complement-mediated functions were grossly deficient. The concentrations of serum complement components were normal except for that of C3 (β,γ-globulin), which was less than one-third normal. The bulk of this was in the form of the inactive conversion product, C3b, at all times that the patient's serum or plasma was examined over a two-year period. Addition of small amounts of normal serum, but not purified C3, to the patient's serum improved all complement-mediated functions in vitro. This disorder, which may represent an inborn deficiency of a protein necessary for C3 stability in vivo and in vitro, is detected by the lowered serum C3 concentration and a positive non-gamma (C3) Coombs antiglobulin test.

THERE are well established associations between increased susceptibility to infection and disorders of the immunoglobulins,1,2 of the cellular mediators of delayed hypersensitivity3 and of the cells of the phagocytic system.4,5 Since complement is known to participate or has been strongly suspected of playing an important part in several phases of mammalian defense against bacterial invasion, it seemed reasonable to suppose that severe deficiencies of one or more complement components would be associated with increased susceptibility to infection. It was quite surprising, therefore, to find no such tendency in persons with a hereditary deficiency of C26,7 or with an inherited partial deficiency of C3,8 in mice deficient in C59, C1 in rabbits deficient in C6.10

In this report we present the clinical and laboratory findings in the case of a young man who has had repeated infections, primarily with pyogenic organisms, since the first year of life, and in whom all known aspects of immunity were intact except for those mediated by the complement system. The results of special studies carried out to define the molecular defects and the pathophysiologic aspects of his disease will be reported elsewhere.

CASE REPORT

T.J., a 25-year-old laborer, had been admitted to the Boston City Hospital on 28 occasions since infancy. The first of these was at the age of 1 year for hemorrhagic measles. Several months later, he was admitted for the first of 11 admissions for acute otitis media or mastoiditis or both. Bacteriologic studies during these episodes revealed infection with Staphylococcus aureus, Proteus vulgaris (Bacillus proteus) or Pseudomonas aeruginosa (Bacillus pseudomonas). Five admissions were for elective otorhinolaryngologic surgical procedures. In addition, the patient was hospitalized for bronchopneumonia, left-lower-lobe pneumonia, Haemophilus influenzae pneumonia, inguinal abscess, acute sinusitis (on 2 occasions), diphtheritic posterior auricular abscess, skin infection and septicemia due to beta-hemolytic streptococcus and meningococci. In all cases of acute infection, there was prompt response to antibiotic therapy.

Other items in the past medical history included tonsillectomy and adenoidectomy at 3 years of age, an undiagnosed episode of fever and kidney pain and swelling at the age of 6 years and scarlet fever on a separate occasion. The patient stated that urticaria developed on exposure to water, either warm or cold, and urticaria was observed to follow a hot shower during one admission to the hospital. There was no family history of increased susceptibility to infection.

On physical examination the patient appeared to be in good health, was slightly obese and exhibited euthyroid features. Other abnormalities included a mastoidectomy scar and thickened tympanic membranes. His hearing was diminished bilaterally. Examination of the genitilia revealed a small penis, a normal male escutcheon and 1 normal-sized and 1 firm, pea-sized testicle. There were no detectable abnormalities of the heart, lungs, liver, spleen or lymph nodes.
The patient was of subnormal intelligence and could not read or write.
Urinalysis failed to disclose any abnormality; the blood urea nitrogen was 15 mg per 100 ml, and creatinine clearance was 142 ml per minute. Other laboratory examinations disclosed a hemocrit of 43 per cent, a white-cell count of 6000, with a normal differential, but an increased percentage of nuclear appendages in polymorphonuclear leukocytes. A platelet count and coagulation studies (kindly performed by Drs. Herbert Strauss and Richard Aster), including a euglobulin lysis time, thromboplastin generation time, thrombin time, partial thromboplastin time and assays for factors V, VII, VIII and X, gave normal values. The reticulocyte count was 1.0 per cent. The patient’s red cells gave a strong positive reaction with a Coombs antiglobulin reagent specific for C3 but a negative reaction with a reagent specific for IgG. Nevertheless, there was no evidence of hemolytic anemia. The patient’s serum contained agglutinins for latex particles, but antinuclear antibodies were not detected, lupus-erythematosus preparations were negative on several occasions and cold-agglutinin titers were normal.

The patient’s peripheral blood karyotype, determined in the Clinical Genetics Laboratory, Children’s Hospital Medical Center, revealed a chromosome number of 47 in 21 of 25 metaphase plates and an XXY pattern consistent with Klinefelter’s syndrome. The response of the patient’s peripheral blood lymphocytes to phytohemagglutinin was normal.

METHODS

Immunoglobulin Determinations

The serum concentrations of the major immunoglobulins were determined by radial immunodiffusion using commercially available plates and standards (Hyland Laboratories, Los Angeles, California). The concentration of IgD was estimated semiquantitatively by immunoelectrophoresis with an antiserum to IgD kindly provided by Dr. William Terry, of the National Immunoglobulin Reference Center, Arlington, Virginia.

Humoral-antibody Response

The patient’s ability to produce serum antibody was tested by the subcutaneous injection of 0.5 ml of fluid tetanus toxoid obtained from the Massachusetts State Biological Laboratories, Boston, Massachusetts. Serum samples were collected before and at intervals for two weeks after immunization. Hemagglutination titers, which were determined in these samples with the use of tanned horse red cells coated with tetanus toxoid, were compared with the titer produced by a standard antiserum provided by the Massachusetts State Biological Laboratories.

Delayed Hypersensitivity

To test delayed hypersensitivity, 0.1 ml each of solutions of monilia antigen (Hollister-Stier Laboratories, Yeadon, Pennsylvania) and mumps antigen (Eli Lilly and Company, Indianapolis, Indiana) were injected intradermally. Induration and erythema were measured at 24 and 48 hours.

Complement Concentrations

Serum hemolytic complement was estimated by a micromodification of the method of Kabat and Mayer. The classic complement components were measured by reagent titrations except that purified C3b was added to the R4. Quantitations were performed by an electroimmunochemical technic, nephelometry or radial immunodiffusion using monospecific antisera for C2, C3, C4, C5 and C6. Dr. Martin Klemperer provided us with rabbit antihuman C2. Radial immunodiffusion plates and standards for the assay of C5 were kindly supplied by Dr. Peter Kohler, of Denver, Colorado, and rabbit antiserum to C6 was generously donated by Dr. Peter Lachmann, of Cambridge, England.

Complement-Mediated Functions

Serum bactericidal activity was assayed by means of a turbidometric method with smooth strains of Salmonella newport and Escherichia coli. Results were expressed as a change in per cent transmission at 580 nm, with time of incubation at 37°C.

Chemotaxis generated by the patient’s serum for normal peripheral blood leukocytes was measured in a modified Boyden chamber. Chemotactic factors were generated by the addition of bovine or human serum albumin and rabbit antialum with the test serum. Results were expressed as per cent of polymorphonuclear leukocytes that had migrated to the far side of a 3-μ pore diameter filter (Millipore Corporation, Bedford, Massachusetts) compared with a normal serum control.

Enhancement of the phagocytosis of Type II Diplococcus pneumoniae by serum in the presence of rabbit antibody was measured with the use of nitroblue tetrazolium as previously described.

Electrophoretic Methods

Agarose electrophoresis was performed as outlined by Laurell and Niléhn. Antigen-antibody crossed electrophoresis was used with monospecific antiserum to C3. The patient’s plasma was separated promptly from blood collected into EDTA and analyzed immediately or was stored at −80°C and thawed immediately before analysis. Such samples are termed “fresh EDTA plasma.”

RESULTS

The concentrations of the major immunoglobulins per 100 ml of serum were as follows: IgG, 915 mg; IgA, 475 mg; and IgM, 135 mg. The ranges of concentrations in normal subjects were 600 to 1500, 30 to 200 and 50 to 200 mg per 100 ml, respectively. On immunoelectrophoresis using an antiserum prepared to IgD, this immunoglobulin was found to be present in approximately normal concentration in the patient’s serum.

After the subcutaneous injection of tetanus toxoid, the titer of anti-tetanus toxoid rose from 0.25 to more than 8.0 hemagglutinating U per milliliter by 14 days, constituting a normal response. Positive skin reactions were obtained on intradermal injection of mumps and monilia antigens (7 mm and 12 mm of erythema and induration). The nitroblue tetrazolium test for chronic granulomatous disease...
TABLE 1. Complement Concentrations in Serum.

<table>
<thead>
<tr>
<th>Subject of Test</th>
<th>Reagent Titration</th>
<th>Immunocchemical Determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C₅a</td>
<td>C₁</td>
</tr>
<tr>
<td>Patient</td>
<td>13.2</td>
<td>1536</td>
</tr>
<tr>
<td>Normal control</td>
<td>32.45</td>
<td>1024-2048</td>
</tr>
</tbody>
</table>

* Total immunochemically reactive C₃: concentration of protein with electrophoretic mobility of active C₃ approximately 8 mg per 100 ml.

Gave normal results with the use of the patient’s peripheral blood leukocytes and latex particles. Having tested the known mediators of host defense and finding them unimpaired, we next turned to the complement system and its functions. Table 1 presents the results of the determinations of hemolytic complement and its components. It is evident that hemolytic complement was markedly reduced in the patient’s serum. However, the concentrations of C₁, C₂, C₄, C₅ and C₆ were normal. Of the components tested, only C₃ showed appreciable reduction on immunocchemical estimation. The fact that the reagent titration for the classic third component of complement was essentially normal reflects the insensitivity of this reagent to C₃ and suggests that the late-acting components, C₇, C₈ and C₉ were normal in concentration.

On agarose electrophoresis (Fig. 1) of the patient’s serum, the protein band corresponding to C₃ (β₂-globulin) in the electrophoretic pattern of the normal serum was absent. This indicated an even greater deficit in C₃ than the immunocchemical data suggested. When the patient’s fresh EDTA plasma was analyzed in antigen-antibody-crossed electrophoresis using an antisera to purified C₃, it was seen that approximately three fourths of the immunocchemically reactive C₃ was in the form of its inactive conversion products, C₃b (Fig. 2). Thus, the concentration of protein with the electrophoretic mobility of native, functionally active C₃ was only 7 or 8 mg per 100 ml (normal range, 100 to 200 mg per 100 ml). This type of antigen-antibody-crossed electrophoresis pattern, with most of the C₃ in a form with the mobility of inactive conversion product, was observed in all samples from the patient over a two-year period at

**Figure 1.** Agarose-Gel Electrophoresis at pH 8.6, Ionic Strength 0.05, Barbituric Buffer with 0.0018 M Calcium Lactate.

The origin is indicated (O) as are the positions of the anode (+), cathode (-), albumin (Alb) and C₃. The pattern of the patient's fresh serum is on top and that of a normal serum below. There is no visible C₃ band in the pattern of the patient's fresh serum.

**Figure 2.** Antigen-antibody-crossed Electrophoresis Using Monospecific Antiserum to C₃.

The buffer conditions are those described in Figure 1. The anode for the initial separation was on the left and for the second separation at the top. The positions of C₃ and the inactive conversion product, C₃b, are shown at the top of the figure. Strips of gel adjacent to those used for the second electrophoresis were stained and placed below the antigen-antibody-crossed electrophoresis patterns for markers. The visible bands in these strips are identified at the bottom of the figure as alpha₂ (α₂-macroglobulin and haptoglobin), Tf (transferrin), Lp (β-lipoprotein, which appears as a double band), C₃ and Fib (fibronogen). The top pattern is of fresh EDTA plasma from the patient, and the bottom pattern of fresh normal serum. It will be seen that C₃ is very greatly reduced in the patient's plasma and that the bulk of the C₃ is in the form of C₃b, not found in the normal serum.
times when he was free of infection as well as when he had active infection. The C3 in the fresh EDTA plasma of nine other patients with Klinefelter's syndrome was normal in concentration, and no conversion products were seen in antigen-antibody-crossed electrophoresis.

When tested for a number of complement-mediated functions in vitro, the patient's serum was grossly deficient, as shown in Figure 3 and Tables 2, 3, 4. Thus, bactericidal activity for gram-negative organisms, chemotaxis for normal leukocytes and the enhancement of phagocytosis by normal leukocytes of pneumococci were all greatly impaired. The addition (to the patient's serum) of small volumes of normal serum, themselves ineffective in these assays, improved all these functions. The addition of purified C3 to the patient's serum to normal serum concentration, however, afforded no important improvement.

**Table 2. Restoration of Hemolytic Complement In Vitro.**

<table>
<thead>
<tr>
<th>Patient's Serum</th>
<th>Purified C3</th>
<th>Normal Serum</th>
<th>CH₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml</td>
<td>mg</td>
<td>ml</td>
<td>U/ml</td>
</tr>
<tr>
<td>1.00</td>
<td>–</td>
<td>–</td>
<td>13.2</td>
</tr>
<tr>
<td>1.00</td>
<td>1.3</td>
<td>–</td>
<td>15.2</td>
</tr>
<tr>
<td>1.00</td>
<td>–</td>
<td>–</td>
<td>16.4</td>
</tr>
<tr>
<td>0.99</td>
<td>–</td>
<td>0.01</td>
<td>20.9 (16.5)*</td>
</tr>
<tr>
<td>0.95</td>
<td>–</td>
<td>0.05</td>
<td>21.8 (17.1)</td>
</tr>
<tr>
<td>0.90</td>
<td>–</td>
<td>0.10</td>
<td>27.5 (17.8)</td>
</tr>
<tr>
<td>0.75</td>
<td>–</td>
<td>0.25</td>
<td>30.9 (19.8)</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>1.00</td>
<td>30.0</td>
</tr>
</tbody>
</table>

*Note: in parentheses are CH₅₀ U/ml expected from simple addition of CH₅₀ U contained in specified volumes of 2 serum samples.

**Table 3. Restoration of Chemotaxis for Normal Leukocytes In Vitro.**

<table>
<thead>
<tr>
<th>Patient's Serum</th>
<th>Purified C3</th>
<th>Normal Serum</th>
<th>Chemotaxis Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml</td>
<td>mg</td>
<td>ml</td>
<td>% normal control</td>
</tr>
<tr>
<td>0.40</td>
<td>–</td>
<td>–</td>
<td>15.5</td>
</tr>
<tr>
<td>0.40</td>
<td>–</td>
<td>0.01</td>
<td>39.0</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>0.01</td>
<td>0</td>
</tr>
<tr>
<td>0.40</td>
<td>–</td>
<td>–</td>
<td>6.0</td>
</tr>
<tr>
<td>0.40</td>
<td>0.52</td>
<td>–</td>
<td>5.0</td>
</tr>
</tbody>
</table>

**Discussion**

The observations in this report provide evidence that our patient has a form of increased susceptibility to infection not previously recognized. No defects could be demonstrated in immunoglobulin concentrations in serum, in antibody formation, in the development of delayed hypersensitivity or in the ability of his white cells to ingest antigenically inert particles in the absence of serum.

Gross abnormalities were demonstrated in hemolytic complement, bactericidal activity for smooth gram-negative organisms, chemotaxis for polymorphonuclear leukocytes and enhancement of the phagocytosis of pneumococci by serum. It appears that C3 was uniquely abnormal in this patient's serum, and the remaining eight components of complement were normal and unaffected, both by reagent titrations of complement components and by immunochromic estimation. The abnormality of C3 was complex but was manifested by continuous inactivation and conversion in vivo of C3 to C3b (C3i). The evidence for this inactivation was the constant presence in the patient's fresh serum or plasma of large amounts of the conversion product. We have demonstrated, and others have confirmed, that the conversion product is much more rapidly cleared from the plasma than is C3 itself. In a group of patients with various forms of nephritis and with other diseases associated with abnormalities of complement, we found circulating conversion product only in three of eight patients with acute glomerulonephritis and then only when the patients' plasma was obtained within the first 24 to 48 hours from the onset of symptoms. Both the absolute concentration of converted C3 and the proportion between C3b (or C3c) and C3 were far greater in the present patient than

**Table 4. Restoration of Enhancement of Phagocytosis.**

<table>
<thead>
<tr>
<th>Patient's Serum</th>
<th>Purified C3</th>
<th>Normal Serum</th>
<th>OD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml</td>
<td>mg</td>
<td>ml</td>
<td></td>
</tr>
<tr>
<td>0.08</td>
<td>–</td>
<td>–</td>
<td>0.061</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>0.08</td>
<td>0.366</td>
</tr>
<tr>
<td>0.08</td>
<td>0.096</td>
<td>–</td>
<td>0.056</td>
</tr>
<tr>
<td>0.08</td>
<td>0.192</td>
<td>–</td>
<td>0.063</td>
</tr>
<tr>
<td>0.08</td>
<td>–</td>
<td>0.02</td>
<td>0.165</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>0.02</td>
<td>0.072</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.070</td>
</tr>
</tbody>
</table>

*Optical density at 515 nm of nitroblue tetrazolium reduced during phagocytosis.
in the patients with nephritis. There are other differences. The conversion in vivo of C3 in the patients with nephritis most probably reflects activation of C1 by antigen-antibody complexes, with subsequent activation of C4 and C2, which then in turn activate and convert C3 in the plasma. The presence of converted C3 in the plasma of these patients is, furthermore, a transient event, whereas it has probably been lifelong in the patient described in this report. The fact that other complement components were unaffected in the present patient suggests either that there is an alternative physiological mechanism for the inactivation of C3 that bypasses the first three complement components and fails to result in activation of the last five components or that there is normally some activation of the entire complement sequence and the activation and inactivation of C3 is unusually pronounced in this patient.

Since it was shown that the addition of purified C3 to the patient's serum did not restore hemolytic complement activity or other complement-mediated functions, his defect is not simply the result of a deficit in C3. The definite restoration of these functions by small amounts of normal serum suggests that there is a deficiency of one or more substances in the patient's serum that are probably not complement components. The same experiments ruled out the presence in the patient's serum of an abnormal or abnormally increased level of a complement inhibitor. The most likely explanation for the findings in this patient and his serum is that he has an inborn defect of an inhibitor of some protease that has C3 as its substrate.

At the time of the original report on this patient,28 Miller and his co-workers29 presented an account of a familial disorder characterized by increased susceptibility to infection with gram-negative organisms and Staph. aureus and impaired phagocytosis of yeast. We and Dr. Miller have exchanged serum samples, and it is now clear that the two defects are quite different and distinct. Serum from his affected family member corrected the defect in phagocytosis in our patient's serum to the same extent as normal serum did. His patient's serum produced normal enhancement of phagocytosis of the pneumococcus in our system. Consistent with these findings are those of Miller's28 when he examined our patient's serum, which restored the defect of his patient's serum in the enhancement of phagocytosis of yeast.

It should be stressed that defective enhancement of pneumococcal phagocytosis in vitro has been found in patients with inherited partial C3 deficiency,9 with acquired C3 deficiency as in progressive glomerulonephritis34 and with hereditary angioneurotic edema in whose sera C4 and C2 are reduced in concentration.20 None of these patients show undue susceptibility to infection. In addition, small amounts of these sera are as effective as normal serum in restoring complement-mediated functions to the present patient's serum in vitro.

We are indebted to Miss Lillian Watson for technical assistance, to Dr. Charles E. McCall for help in setting up the chemotaxis assay, to Dr. Martin Klemperer for performing the reagent titrations of complement and to Drs. Daniel Federman and Alan Perlmutter for the opportunity to study their patients with Klinefelter's syndrome.

REFERENCES


The New England Journal of Medicine
Downloaded from nejm.org at Harvard Library on October 16, 2014. For personal use only. No other uses without permission.
From the NEJM Archive. Copyright © 2010 Massachusetts Medical Society. All rights reserved.
A FAMILIAL DEFICIENCY OF THE PHAGOCYTOSIS-ENHANCING ACTIVITY OF SERUM RELATED TO A DYSFUNCTION OF THE FIFTH COMPONENT OF COMPLEMENT (C5)*

MICHAEL E. MILLER, M.D., AND ULF R. NILSSON, M.D.

With the Technical Assistance of Kenneth A. Myers

Abstract A patient, previously described, was found to have increased susceptibility to bacterial infections related to a deficiency of serum enhancement of in vitro phagocytosis. The same deficiency affected the patient’s mother and 15 other relatives. The deficiency was shown to involve a dysfunction of the fifth component of complement (C5) by the fact that the phagocytosis-enhancing activity of the mother’s serum was restored to normal by the addition of highly purified C5. Also, serum from mice with a genetic deficiency of C5 indicated poor enhancement of phagocytosis, and the addition of highly purified C5 to the C5-deficient mouse serum restored phagocytosis-enhancing activity. Finally, the addition of C5-deficient mouse serum to the mother’s serum failed to improve the phagocytosis-enhancing effect of the maternal serum, whereas the addition of small amounts of normal mouse serum did.

In view of the patient’s clinical susceptibility to primarily gram-negative bacteria, the in vitro dysfunction of C5 may be related to impaired in vivo inactivation of such organisms.

RECENTLY, Miller and his co-workers described a family with an inherited defect of humoral mediation of phagocytosis. The proband was a seven-month-old female infant who was referred to the Children’s Hospital of Philadelphia because of failure to thrive, severe eczematoid dermatitis and repeated local and systemic infections from a variety of gram-negative bacteria and coagulase-positive staphylococci. A deficiency of phagocytosis-enhancing function of the serum was found by an in vitro assay of phagocytosis. The assay is based upon quantitation of uptake of yeast particles by human polymorphonuclear leukocytes (PMNS), and permits separate evaluation of the cellular and humoral components of the phagocytic process. Leukocytes from the patient or from normal controls showed no deficiency in yeast ingestion when incubated in the presence of pooled, normal plasma (or serum). When leukocytes from the patient or normal controls were incubated in the presence of the patient’s plasma, however, they showed markedly diminished ability to ingest yeast particles, as they also did when Staphylococcus aureus was the particle being ingested. A humoral, rather than a cellular, defect was thus defined. A similar defect was found in a number of relatives, including the mother and both maternal grandparents. It was found that infusion of fresh or fresh-frozen plasma corrected the impairment of the phagocytosis-enhancing function (in vitro) and was regularly followed by clinical improvement of the patient. Initial studies demonstrated that the defect in phagocytosis resulted from a deficiency of a serum factor rather than from the presence of an inhibitor, and that this factor was heat labile.

Although a primary defect in the complement system was thought not to be involved, further studies on the nature of the deficient factor have now revealed that the dysfunction in this family involves the fifth component of complement (C5). Since a deficiency of C5 has not previously been shown to result in impaired phagocytosis in vitro, these data have potential implications extending beyond the understanding of the disease that led to their discovery.

MATERIALS AND METHODS

Since the patient had received numerous plasma infusions, for these studies serum was obtained from her mother, who was previously known to have the same deficiency of in vitro phagocytosis enhancement.

Chemotaxis

The method employed was a modification of the Boyden assay as described in detail elsewhere. In summary, 0.5 ml of polymorphonuclear leukocyte

---

*From the departments of Pediatrics and Medicine, University of Pennsylvania School of Medicine and the Children’s Hospital of Philadelphia (address reprint requests to Dr. Miller at the Department of Pediatrics, Hospital of the University of Pennsylvania, 3600 Spruce St., Philadelphia, Pa. 19104).

Aided by a grant from the John A. Hartford Foundation and by grants FR 00240, HE 10-907 and AI 00319 from the United States Public Health Service (Dr. Miller is the recipient of a career development award from the United States Public Health Service [1 KO 4 HD 25 978-01]).
