Objective: To define techniques used for complement measurements and examine the clinical relevance of alterations of complement determinations in disease.

Data Sources: Data have been assembled from the authors’ research, original articles, and reviews, as well as chapters and complete books on complement.

Study Selection: Studies were chosen for inclusion by the opinions of the authors, relevant complement reviews, publications, and books.

Results: Complement has been shown to possess approximately 31 proteins, some of which are enzymes (C1r, C1s, C2, factor B, factor D), some cofactors, some inhibitors or inactivators, and others composed of membrane-integrated proteins. All of the complement proteins have been purified, and many of the respective genes have been identified. The complement cascade is a dual-edged sword, causing protection against bacterial and viral invasion by promoting phagocytosis and inflammation. Pathologically, complement can cause substantial damage to blood vessels (vasculitis), kidney basement membrane and attached endothelial and epithelial cells (nephritis), joint synovium (arthritis), and erythrocytes (hemolysis) if it is not adequately controlled.

Conclusions: Definitive evidence is available that complement-mediated tissue destruction occurs after immune complex injury in the kidney and lung and may be important in lupus erythematosus and adult respiratory distress syndrome. Future studies on complement receptor structure and function may provide clues to treat effectively lupus, hemolytic anemias, and nephritis. In addition, gene therapy and antibody therapy need further refinement to treat immunodeficiency diseases.


Off-label disclosure: Drs. Glovsky, Ward, and Johnson have indicated that this article does not include the discussion of unapproved/investigative use of a commercial product/device.

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INTRODUCTION
Since the discovery of complement by Jules Bordet more than 100 years ago, the importance of the complement system in producing lysis of bacteria and protecting humans and experimental animals against infectious microorganisms has been appreciated.1–9 Within the past 30 years, complement has been shown to possess approximately 31 proteins, some of which are enzymes (C1r, C1s, C2, factor B, factor D), some

cofactors, some inhibitors or inactivators, and others composed of membrane-integrated proteins. All of the complement proteins have been purified, and many of the respective genes have been identified.1 Other proteins of the complement system include the receptors for C1q, C3a/C4a, C5a, CR1, CR2, and CR3 (C3b, C3b, and C3d receptors). In addition, membrane components (decay-accelerating factor, CD55 and CD59, and membrane inhibitor of C8 and C9 insertion) are important regulating proteins. The complement cascade is a dual-edged sword, causing protection against bacterial and viral invasion by promoting phagocytosis and inflammation. Pathologically, complement can cause substantial damage to blood vessels (vasculitis), kidney basement membrane and attached endothelial and epithelial cells.
(nephritis), joint synovium (arthritis), and erythrocytes (hemolysis) if it is not adequately controlled. This review defines techniques used for complement measurements and examines the clinical relevance of alterations of complement determinations in disease. Data have been assembled from the authors’ research, original articles, and reviews, as well as chapters and complete books on complement.

COMPLEMENT ACTIVATION MECHANISMS
There are 3 currently known complement activation mechanisms (Fig 1): (1) the classical pathway, (2) a recently described pathway (the mannose-binding lectin pathway), and (3) the alternative pathway.

Immune complexes, apoptotic cells, or C1q bound to its ligand can activate the classical pathway. On activation, C1r and C1s are converted from proenzymes to activated enzymes and cleave their natural substrates C4 and C2 to C4a and C4b and C2a and C2b, respectively. C4b and C2a form C4b2a (C3 convertase of the classical pathway), which splits C3 to C3a and C3b. C3b combines with C4b2a to form C4b2a3b (C5 convertase), which cleaves C5 to C5b and C5a. This leads to formation of the C5b, C6, C7, C8, and C9 membrane attack complex (MAC).

Mannose-binding lectin, when bound to mannose residues on microbial surfaces, can interact with 2 serine proteases: MASP-2 and MASP-1. MASP-2 cleaves and inactivates C4 and C2 to form C4b2a. MASP-1 may cleave C3 directly, promoting the formation of C4b2a3b, the C5 convertase of the classical pathway.

The alternative pathway can be activated in the absence of antibody by insoluble polysaccharides, yeast cell walls, and aggregated IgA and IgE at high concentrations. Factor D, a serine protease, cleaves factor B to Ba and Bb. The larger fragment of factor B combines with the large fragment of C3,
C3b, to form the alternative pathway C3 convertase, C3bBb. Properdin stabilizes the C3 convertase by binding to the complex, PC3bBb. C3bBb then can further cleave C3 to form additional C3b molecules, some of which combine with C3bBb to form C3bBbC3b, the C5 convertase of the alternative pathway, which cleaves C5 to C5a and C5b. Thus, the classical pathway and the mannose-binding lectin pathway, as well as the alternative pathway, converge on C3 to initiate the formation of C3b, the complement factor, when bound to microorganisms, which initiates phagocytosis and destruction of the invading microbe. C3a, a proinflammatory anaphylatoxin, is also generated.

The MAC complex (C5b-C9) and C5a (the most potent anaphylotoxin) are generated by enzymatic cleavage of C5 to C5b and C5a. Table 1 lists alterations in the complement activation pathways and some of the diseases associated with activation mechanisms.

Activation of the classical complement pathway such as seen in immune complex disease associated with DNA–anti-DNA antibodies is often associated with low CH50, low C4, and low C3. Alternative pathway activation of factor B and C3 is seen in endotoxin shock syndromes, as well as in sera of patients with membranoproliferative glomerulonephritis. Also, activation of the classical pathway occurs in the relative absence of the C1 inhibitor as seen in hereditary angioedema (HAE). Fluid-phase activation of the alternative pathway occurs with factor I deficiency, when C3 convertase activity is not controlled. With chronic inflammation that occurs in Reiter syndrome, acute-phase reactants, such as C4, factor B, and haptoglobin, may be increased. Young children with low mannose-binding lectin levels frequently are infected with bacterial and yeast pathogens. The decreased levels of CH50 and C4 reflect classical pathway activation, whereas decreased C3 and factor B levels with normal C4 levels are indicative of alternative pathway activation. Deficiencies of the regulator proteins C1 inhibitor and factor I are reflected by increased classical and alternative pathway activation, respectively.

CONTROL MECHANISMS OF THE COMPLEMENT SYSTEM

To control activation of the classical pathway, C1 inhibitor binds to activated C1r and C1s and inactivates these enzymes. The binding is in a 1:1 ratio. The C4b2a complex (C3 convertase) is controlled by C4 binding protein, which binds to the complex. C4b is then cleaved by factor I. Under physiologic conditions, the classical pathway control stops the further breakdown of classical pathway components. The alternative pathway C3 convertase (C3bBb) is regulated by factor H, which competes with Bb, the split product of factor B, for the binding site on C3b, forming (C3bH). The C3b is further degraded by factor I to smaller breakdown products. Note the similar control mechanisms of the classical and alternative pathways. The classical and alternative pathway convertases are broken up by C4b binding protein and factor H, respectively.

LABORATORY MEASUREMENT

Complement components can be measured either as proteins or based on their functional activity. Because the proteins that comprise the classical and alternative complement cascades have been isolated in chemically pure form, antibodies have been produced to these proteins in several animal species. These antibodies may be used to quantitate complement components through one of several immunochemical procedures.

In radial immunodiffusion, antibody to a single complement component is incorporated into an agar gel. Test serum is placed in wells cut into the agar. During a subsequent incubation period that lasted 1 to 3 days, the specific complement protein diffuses into the agar, forming a precipitin ring in its interaction with antibody. The diameter of the ring reflects the concentration of complement proteins present in the test serum. The precise amount is determined by comparing the diameter of the precipitin ring formed with standard solutions of complement protein.

Table 1. Complement Profiles in Disease

<table>
<thead>
<tr>
<th>Activation pathway</th>
<th>CH50</th>
<th>C4</th>
<th>C3</th>
<th>Factor B</th>
<th>Related diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical pathway</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>Systemic lupus erythematosus, some immune complex diseases, some cases of urticaria and angioedema</td>
</tr>
<tr>
<td>Alternative pathway</td>
<td>↓</td>
<td>N</td>
<td>↓</td>
<td>↓</td>
<td>Endotoxin shock, membrano-proliferative glomerulonephritis</td>
</tr>
<tr>
<td>Fluid-phase activation of classical pathway</td>
<td>N or ↑</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>Hereditary angioedema</td>
</tr>
<tr>
<td>Fluid-phase activation of alternative pathway</td>
<td>N or ↑</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>C3b inactivator deficiency</td>
</tr>
<tr>
<td>Acute-phase reactions</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>Chronic nonimmune complex diseases such as seen in Table 5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Activation pathway</th>
<th>MBL</th>
<th>MASP-1, -2</th>
<th>Related diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannose-binding lectin pathway</td>
<td>↓</td>
<td>?</td>
<td>Recurrent infections in young children, 6 months to 2 years</td>
</tr>
</tbody>
</table>

Abbreviations: MASP, mannose-binding lectin-associated serine protease; MBL, mannose-binding lectin.
Another assay system uses rate nephelometry. This technique takes advantage of the fact that the interaction of antigen and antibody in solution leads to the formation of a precipitate that deflects a beam of laser light. From the rate of change of “light scattering,” it is possible to determine the amount of antigen (ie, specific complement component) in the test specimen.

Table 2 shows the average concentration of the complement components in normal serum. Note that C3, C4, and factor H are present at the highest concentrations. It is interesting that the total concentration of complement components and inactivators make up 10% of the globulin protein in normal human serum.

Another approach to measuring complement proteins is in terms of their hemolytic activity. The functional activity of the classical complement pathway can be evaluated by measuring the ability of serum to lyse antibody-coated sheep erythrocytes. Sheep erythrocytes are optimally coated with rabbit anti-sheep erythrocyte antibody (amboceptor or hemolysin). Next, various amounts of diluted test serum are added and the mixture is incubated at 37° C. In the presence of complement, antibody-coated cells are lysed; the extent of lysis can be determined by measuring the amount of hemoglobin released in the solution. The CH50 is the most useful complement test in screening for homozygous C1 through C9 deficiencies. The amount of serum required to lyse 50% of a standard concentration of antibody-coated sheep erythrocytes in a standard period is called the CH50 value. All 9 complement components are required for normal hemolytic activity. With homozygous deficiency of C1 to C8, less than 5% CH50 values are present. Homozygous deficiency of C9 usually results in less than 30% CH50, since C9 is not required for hemolysis. Heterozygous component deficiency may be associated with low CH50 values.

The activity of the individual components C1, C4, C2, C3, C5, C6, C7, C8, and C9 can also be determined. Basically, the component to be measured is determined from dilutions of the serum sample. Other components are added in excess, and after appropriate incubation steps, the hemolytic titer of the individual component is calculated. In a similar manner, the activity of the complement inhibitors, C1 inhibitor and C3b inactivator, can also be determined by their ability to inhibit hemolysis of C1 and C3.

To measure complement activity, freshly separated serum or EDTA plasma is required. For accurate measurements, separated serum should be frozen within 1 hour of drawing (at −20° C), or EDTA plasma should be used.

**COMPLEMENT LEVELS AT BIRTH**

Complement component levels in the serum are lower at birth than in adult sera. Among the low components are C1q, C2, C5, C7, C8, and C9. C2 reaches normal adult concentrations by 1 month. Yet the other components take up to 3 years to reach normal adult concentrations. Levels of C3, C4, C6, and factors B, I, and H are low at birth. By 1 year of age, these latter components reach adult levels.

**INBORN ERRORS OF THE COMPLEMENT SYSTEM**

Genetic deficiencies of each of the complement components have been found (Tables 3 and 4). The deficiencies are usually associated with autosomal recessive inheritance. In those patients with homozygous deficiencies of C1, C4, or C2, an increased incidence of systemic lupus-like syndromes has been found. The most common genetic defect in the complement system is C9 deficiency found in Japanese blood donors. Eighteen probands with nearly total absence of C3 have also been previously described. These patients have severe infections with gram-negative and gram-positive mi-
croorganisms. Their serum does not promote bacterial phagocytosis, lyse immune complexes normally, generate chemotactic factors or anaphylatoxins from serum, or destroy certain gram-negative bacteria. There are 2 separate C4 loci that give rise to 2 protein products, C4A and C4B. A deficiency in either the C4A or C4B allele is called Q0 or quantity 0 for the gene product. The absence of either C4A (C4AQ0) or C4B (C4BQ0) is associated with autoimmune disease, usually systemic lupus.3

The remaining genetic deficiencies have been found in 10 to 100 families. Properdin-deficient patients often have life-threatening infectious disease.9,20 Patients with homozygous deficiency of C5 have also experienced recurrent bacterial infections, perhaps related to the inability to provide a sufficient chemotraction (C5a) for leukocytes to remove the offending organisms. Patients with homozygous C5, C6, C7, and C8 deficiencies have been shown to have recurrent Neisseria infections.3 Their serum has less bactericidal activity to gonococci and meningococci than normal serum. The complement system is extremely efficient in providing opsonic signals, lysing erythrocytes, attracting leukocytes, causing increased vascular permeability, inactivating viruses, and killing some gram-negative bacteria. Because of this, those individuals with partial or heterozygous defects are usually not subjected to increased incidence of infectious disease.

As seen in Table 4, complement components are located on 8 separate chromosomes (1, 4, 5, 6, 8, 9, 12, and X).9,20 Systemic lupus erythematosus is associated with deficiencies of C1q, C1rs, C4, C2, and C3. Since these components are located on 4 different chromosomes, it is unlikely that lupus could be caused by immune deficits on each of the chromosomes. More plausible is the theory that an intact complement system is required to control systemic lupus.

Complement components C1, C4, C2, and C3 can break up immune complexes (DNA–anti-DNA).9,20 The inability to control the cleavage of such complexes may be important in the cause of lupus and other immune complex diseases, such as rheumatoid arthritis and glomerulonephritis.

### Table 3. Inborn Errors of the Complement System

<table>
<thead>
<tr>
<th>Components</th>
<th>Inheritance</th>
<th>Associated diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1q</td>
<td>Autosomal recessive</td>
<td>Recurrent bacterial infections</td>
</tr>
<tr>
<td>C1r</td>
<td>Autosomal recessive</td>
<td>Lupus-like rash, glomerulonephritis</td>
</tr>
<tr>
<td>C1s</td>
<td>Autosomal recessive</td>
<td>Systemic lupus, lupus syndrome, healthy</td>
</tr>
<tr>
<td>C4</td>
<td>Autosomal recessive</td>
<td>Lupus</td>
</tr>
<tr>
<td>C2</td>
<td>Autosomal codominant</td>
<td>Lupus, glomerulonephritis, recurrent bacterial infections in childhood, may be healthy</td>
</tr>
<tr>
<td>C3</td>
<td>Autosomal recessive</td>
<td>Recurrent bacterial infections, clinically similar to agammaglobulinemia</td>
</tr>
<tr>
<td>C5</td>
<td>Autosomal recessive</td>
<td>Systemic lupus, recurrent infections</td>
</tr>
<tr>
<td>C6</td>
<td>Autosomal recessive</td>
<td>No unusual disease, gonococcal arthritis nephritis, Neisseria meningitis</td>
</tr>
<tr>
<td>C7</td>
<td>Autosomal recessive</td>
<td>Scleroderma or no disease</td>
</tr>
<tr>
<td>C8</td>
<td>Autosomal recessive</td>
<td>No disease or recurrent Neisseria infections</td>
</tr>
<tr>
<td>C9</td>
<td>Autosomal recessive</td>
<td>No disease</td>
</tr>
</tbody>
</table>

### Table 4. Chromosomal Location of Complement Components or Subcomponents

<table>
<thead>
<tr>
<th>Component</th>
<th>No. of cases</th>
<th>Chromosome location</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1q</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>C1r, s</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>C4</td>
<td>17</td>
<td>6, close to MHC locus</td>
</tr>
<tr>
<td>C2</td>
<td>&gt;100</td>
<td>6, adjacent to factor B</td>
</tr>
<tr>
<td>Factor B</td>
<td>1</td>
<td>6, adjacent to C2</td>
</tr>
<tr>
<td>Factor D</td>
<td>25</td>
<td>X</td>
</tr>
<tr>
<td>Properdin</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>C3</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Factor I</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Factor H</td>
<td>19</td>
<td>9</td>
</tr>
<tr>
<td>C6</td>
<td>&gt;50</td>
<td>5, Near C7</td>
</tr>
<tr>
<td>C7</td>
<td>28</td>
<td>5</td>
</tr>
<tr>
<td>C8</td>
<td>32</td>
<td>1, α-chain and β-chain</td>
</tr>
<tr>
<td>C9</td>
<td>Many (&gt;1,000)</td>
<td>5</td>
</tr>
</tbody>
</table>

Abbreviation: MHC, major histocompatibility complex.

DEFICIENCIES OF COMPLEMENT INACTIVATORS

Hereditary angioedema (HAE) has been recognized for more than a century. It was described by Sir William Osler in the 1880s and by Quincke 30 years earlier. Yet the biochemical understanding that a complement inhibitor (C1 inhibitor) was the chemical defect responsible for HAE occurred more than 4 decades ago.

In 1962, Landerman21 showed that serum from a patient with HAE lacked inhibitory activity for kallikrein, bradykinin, and Hageman factor (factor XII of the clotting system). In 1963, Donaldson and Evans showed that the C1 inhibitor was deficient in serum of HAE. Thus, the C1 inhibitor possesses multiple inhibitory protein interactions with components of clotting (XII, XI), fibrinolysis, plasmin, vascular permeability, kallikrein, and bradykinin.22,23 HAE is the only complement deficiency transmitted as a dominant trait (ie, a single abnormal gene together with a normal gene can produce the deficiency).20 Clinical symptoms of HAE consist of angioedema of the skin and other organs, frequently caused
by trauma and sometimes by viral infections. In the past, approximately 25% of patients died of laryngeal edema, often initiated by dental or pharyngeal surgery. Abdominal pain is caused by swelling of the intestinal mucosa and can be easily observed during an attack by radiographs of the upper gastrointestinal tract as markedly swollen intestinal villi. The attacks last for 48 to 72 hours and subside as C4 and C2 levels fall. Generation of C4b2a, the C3 convertase of the classical pathway, is halted by the C4 binding protein (C4BP). The mechanism of “shutting off an acute attack” is unclear as are the specific vasopermeability factors that cause the swelling and pain. Some factors implicated are C4a, bradykinin, histamine, and a split product of C2 (C2 kinin).  

Diagnosis of HAE  
The characteristic clinical findings plus family history can often pinpoint the diagnosis. Yet the biochemical finding of low C1 inhibitor function makes the diagnosis certain. In the more common variety, type 1 HAE (85% of cases), C1 inhibitor protein is low (<30% of the normal mean) due to a synthetic or regulatory gene defect. Levels of C4 protein, a substrate of C1 esterase, are low in 95% of patients with HAE. For 15% of the cases, a normal or elevated C1 inhibitor protein level is found. This occurs because a structural defect that inhibits function, but not antigenic recognition of the protein, is present and is called type 2 HAE.  

Patients with lymphoma and other carcinomas may have low C1 inhibitor protein and low C4 levels. Markedly diminished C1q levels distinguish this acquired form of C1 inhibitor deficiency from HAE. C1q may be removed by binding to lymphoma or other neoplastic cells as a consequence of complement activation on the cell surface. Another type of acquired angioedema with low C1 inhibitor function is caused by antibody to C1 inhibitor protein that interferes with its function. Transient diminution of C1 inhibitor protein associated with angioedema with normal C1q has also been seen. This is a benign form of disease associated with classical pathway activation.

Treatment of HAE  
Since only a single gene is deficient in HAE, treatment designed to raise the production of the C1 inhibitor has been used. A synthetic impeded androgen, danazol, has been shown to increase the levels of C1 inhibitor and decrease consumption of C4. It has markedly reduced the frequency of attacks in HAE and protects against oral surgery–induced laryngeal edema. Yet androgen treatment has undesirable side effects: reduction in number of menstrual periods and prevention of pregnancy, increase in muscle bulk, and induction of virilization. For these reasons, doses of the impeded androgens are maintained at low levels to reduce clinical symptoms and not to normalize C1 inhibitor levels. Interestingly, C4 protein levels usually return to the normal range with successful prophylaxis. To treat acute attacks of severe angioedema, ε-amino-caproic acid, an inhibitor of plasmin activation, is effective. It is given in large doses (usually 1 g intravenously per hour for a total of 16 g). Both purified C1 inhibitor protein and fresh frozen plasma are also effective to shut off acute attacks. Purified C1 inhibitor protein has not, as yet, been approved for treatment in the United States. A small percentage of patients (2% to 5%) with HAE also manifest autoimmune disease, such as lupus or rheumatoid arthritis.

C3b INACTIVATOR (FACTOR I) DEFICIENCY  
More than 12 patients with homozygous deficiency of factor I have been described. The patients have less than 10% C3, because the C3 convertases are not readily inactivated. Although factor H is present and can break up C3bBb complexes to C3b and Bb, inactivation of C3b is suboptimal. Thus, the amplification control of C3b cleavage is inefficient, allowing further C3 breakdown and levels of C3 in the plasma to fall.

ACQUIRED DISEASE ASSOCIATED WITH ELEVATED COMPLEMENT ACTIVITY  
Table 5 shows several diseases in which either normal or elevated levels of whole complement and several of the components have been repeatedly observed. In general, diseases characterized by chronic inflammation (in the absence of complement fixing circulating immune complexes) are associated with elevated CH50 activity.

ACQUIRED DISEASES ASSOCIATED WITH LOW COMPLEMENT ACTIVITY  
Paramount in understanding the meaning of static complement and component measurements is an appreciation that synthetic and catabolic rates of the individual components affect the component values. In immune complex diseases, such as systemic lupus, increased catabolism of C3 usually occurs and synthesis increases may also be found. Thus, low levels of C3 may reflect increased catabolism, decreased synthesis, or a combination of both. Yet, it appears that in experimental situations and in lupus with high titers of anti-DNA antibody, transient, significant decreases in complement activity precede either increased synthesis or removal of the complexes. In the absence of being able to measure turnover of complement components by most laboratories, serial determinations of complement activity in disease are usually helpful.

Diseases in which circulating immune complexes are found are associated with diminution of CH50 and other
complement components. Frequently, the magnitude of complement depression is related to the activity of the disease, such as systemic lupus and poststreptococcal glomerulonephritis. When the disease remits, complement activity usually returns to normal. As such, measuring serial levels of whole complement or C3 and C4 component activity is useful in assessing the severity of the disease and the response to treatment. The disorders listed in Table 6 are associated with marked depression of CH50 and the C1 through C5 components, indicating primarily classical pathway activation and component consumption. In acute renal allograft rejection, approximately 20% of the rejection crises are associated with a decrease in CH50 and C2 activity. In approximately 5% of patients with urticaria and angioedema, immune complex–associated activation of complement is found. Low complement activity occurs when components are not synthesized at normal rates. Since several components (C3, C6, C9) are synthesized in the liver, marked cirrhosis is associated with low CH50 activity. Membranoproliferative glomerulonephritis of childhood X-linked–type agammaglobulinemia

Table 7. Acquired Diseases Associated with Activation of Specific Complement Pathways

<table>
<thead>
<tr>
<th>Classical pathway activation</th>
<th>Alternative pathway activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryoglobulin IgG-K</td>
<td>Endotoxin shock</td>
</tr>
<tr>
<td>Waldenström macroglobulinemia</td>
<td>Membranoproliferative nephritis</td>
</tr>
<tr>
<td>Lymphosarcoma with 7S IgM</td>
<td>Paroxysmal nocturnal hemoglobinuria</td>
</tr>
<tr>
<td>Radiocontrast-induced anaphylaxis</td>
<td>Renografin- and Cholografin-induced anaphylaxis</td>
</tr>
<tr>
<td>Cellulose membrane (hemodialysis)–induced transient neutropenia</td>
<td>Cardiopulmonary bypass activation of C3</td>
</tr>
</tbody>
</table>

biosynthetic defects of C3. C1 is found in lower concentrations than normal in x-linked agammaglobulinemia. The nature of the control mechanisms for complement component synthesis is as yet unknown.

Table 7 shows that paraproteins in high concentrations and abnormal immunoglobulins are frequently associated with complement activation by the classical pathway. In cases of lymphosarcoma with 7S IgM, a deficiency of the C1 inhibitor is found along with diminution of C1, C4, and C2. Other acquired diseases are associated with preferential activation of the alternative complement pathway. Table 7 also outlines these representative diseases. Recently, radiocontrast dyes, such as Renografin and Cholografin, have been shown to activate the classical and alternative complement pathways and, thus, to produce urticaria, angioedema, and hypotension.

**COMPLEMENT ACTIVITY IN BODY FLUIDS**

Like the serum, complement diminution in pleural, pericardial, and synovial fluids (<30% of normal serum activity) has been found in lupus. In contrast, serum complement activity is usually normal or high in seropositive rheumatoid arthritis, yet synovial and pleural values are markedly lowered. The nonrheumatoid diseases, including neoplasms, degenerative diseases, embolic phenomenon, and chronic infections, are associated with relatively high CH50 (>30% of serum activity) and component levels in exocrine fluids. Blister fluids reflect complement activation, and in pemphigus vulgaris and bullous pemphigoid, complement is bound to the skin lesions, where complement components are found to be diminished within the fluid. Likewise, in both discoid lupus and systemic lupus, complement components are bound in the dermal vessels. C3 complement binding in subepithelial basement membrane, intercellular cement, and basement membrane is a useful adjunct in the differential diagnosis of pemphigoid, pemphigus vulgaris, and dermatitis herpetiformis, respectively. Figure 2 shows the dermal-epidermal separation in bullous pemphigoid and the deposition of C3 in the dermal-epidermal junction by immunofluorescence.

**COMPLEMENT ACTIVITY IN RENAL DISEASE**

Both the serum levels of CH50 and complement components and binding of complement components to or within the glomerular basement membrane have provided insight into the pathogenesis of acute renal disease. Serum complement levels, including C4, C2, and C3, are low in most patients with lupus nephritis and in the early stages of poststreptococcal glomerulonephritis. In the latter disorder, early components C4 and C2 return to normal before C3. Membranoproliferative glomerulonephritis is usually associated with very low levels of C3; normal levels of C1, C4, and C2; and the presence of an autoantibody to C3 convertase. Figure 3 depicts neutrophil accumulation and C3 deposition in acute glomerulonephritis.

Frozen sections of kidney stained with immunofluorescent antisera to complement components show binding of C3 in a “lumpy-bumpy” fashion in subepithelial deposits in lupus and
poststreptococcal nephritis as shown in Figure 3B. Striking deposits of C3 are also found in kidney biopsy specimens from patients with membranoproliferative nephritis. In contrast, a linear, less intensive deposition of C3 along the basement membrane is seen in most patients with Goodpasture syndrome. In chronic glomerulonephritis, C3 deposits may sometimes be seen; their presence or absence has less diagnostic significance. In nephrotic syndrome, C3 deposition in the glomeruli is not characteristically found.

ANAPHYLATOXINS (C3a AND C5a): POSSIBLE MEDIATORS OF ALLERGIC DISEASE
In 1910, Friedberger described substances (anaphylatoxins) that could be generated by immune complexes in normal guinea pig serum. After intravenous injection in guinea pigs, high doses of the anaphylatoxin produced lethal shock and bronchospasm. More than half a century elapsed before the chemical nature of anaphylatoxins was defined. Molecular biology advances in the last 20 years have revealed the chemical structure of C3a, C4a, and C5a and enabled purified reagents to be produced by molecular cloning. In potency, C5a is 10 to 100 times more potent than C3a, and C5a is 1,000 times more potent than C4a. When the C-terminal arginine is enzymatically removed by carboxypeptidase, C3adesarg and C4adesarg are completely inactive; however, C5adesarg retains 10% to 50% of its biologic activity.

ANAPHYLAXIS AND LUNG INJURY
Anaphylaxis or vascular collapse and death follow intravenous or intratracheal injection of C5a into rabbits and rats, respectively. The target organ of C5a and C3a in the guinea pig and the rat is the lung; thus, after complement activation in the rat, increased vascular permeability (edema) and alveolar hemorrhage are evident. Pulmonary hypertension can
be produced by C3a injection intravenously into isolated lungs and is mediated by the release of thromboxane from the lung.\textsuperscript{29} Human counterparts of lung injury due to complement have not been described. Possible diseases include adult respiratory distress syndrome and hypersensitivity pneumonitis induced by fungal antigens such as \textit{Aspergillus fumigatus}.

**POSSIBLE MECHANISMS OF URTICARIA, ANGIOEDEMA, AND ASTHMA**

C3a and C5a, when injected into the skin, produce a wheal-and-flare response similar to immediate hypersensitivity responses found with allergy extracts in the skin. Also, C5a and to a lesser extent C3a induce histamine release with human basophils.\textsuperscript{30} When incubated with human bronchial tissue, C3a causes bronchial constriction that is associated with leukotriene release from the bronchial sample.\textsuperscript{31} C5a causes bronchial constriction as well. Chemotaxis of eosinophils and neutrophils in the airways of rats is likely to be caused by C3a and C5a, as well as other chemokines.

Radiocontrast agents, such as Cholografin and Renografin, produce hypotension, itching, and hives most likely by generation of anaphylatoxins (C3a and C5a). Also, bee venom–induced anaphylaxis has been shown to be associated with both IgE mechanisms and C3a production.

**ALLERGENS**

When added in small amounts to human serum, dust and \textit{Aspergillus} extract generate C3a, C4a, and C5a in maximal amounts. House dust mite and rye grass allergens produce significantly less anaphylatoxins in normal human serum.\textsuperscript{32} Although quantitative amounts of allergens necessary to produce anaphylatoxins in the blood may rarely be achieved, in the microenvironment of the nose and airways, nanogram quantities of house dust and mold are often found.

**COMPLEMENT IN SEPSIS**

There is accumulating evidence in sepsis (in both humans and animals) that complement activation has occurred. CH\textsubscript{50} levels decline and C3a and C5a levels are increased in sera. The content of the receptor for C5a (C5aR) on blood neutrophils is decreased due to internalization of C5a-C5aR complexes. Experimentally, it has been shown in rats and mice with sepsis that interaction of C5a-C5aR occurs on neutrophils. These observations suggest that in sepsis there is consumptive depletion of CH\textsubscript{50} and excessive generation of C5a, which leads to signaling defects in blood neutrophils. Such defects in innate immunity produce increases in microbial growth and could result in death of the human or experimental rodents.\textsuperscript{33}

**PAROXYSMAL NOCTURNAL HEMOGLOBINURIA**

Paroxysmal nocturnal hemoglobinuria (PNH) is a blood disease caused by the lack of one or more glycosylphosphatidylinositol–linked proteins that protect red cells and platelets from lysis by the complement MAC (C5b, C6, C7, C8, C9). Two of these proteins are CD55 and CD59. CD55 inhibits C3 convertases, and CD59 blocks the assembly of MAC (C5b-9) by interacting with C8 and C9. The lack of CD55 and CD59 is associated with clinical symptoms of PNH; black urine due to lysis of red blood cells and free hemoglobin in the urine; thrombosis of unknown origin, possibly related to platelet lysis; and fatigue and intermittent episodes of abdominal pain.\textsuperscript{34} Recently, in preliminary studies, the use of a humanized anti-C5 monoclonal antibody, eculizumab, has markedly improved the need for transfusions and quality of life in 11 patients with PNH. Eculizumab, by binding to C5, inhibits the formation of the MAC complex and thus the hemolytic events. The commercial availability of eculizumab should improve the symptoms of PNH and is likely to benefit those patients in whom C5 activation is related to disease severity, such as gram-negative sepsis.

**CONCLUSION**

Within the last 30 years, most of the relevant complement proteins have been cloned and sequenced. Definitive evidence is available that complement-mediated tissue destruction occurs after immune complex injury in the kidney and lung and may be important in lupus erythematosus and adult respiratory distress syndrome. It is hoped that within the next decade knowledge of complement receptor structure and function may provide clues to treat effectively lupus, hemolytic anemias, and nephritis. Gene therapy and antibody therapy are available yet need further refinement to treat immunodeficiency diseases. In addition, gene replacement for homozygous deficiency of C1q, C4, C2, and C3 may be available. We have come a long way in the scientific clarification of complement mechanisms. Yet, the most exciting discoveries of cures for complement-related diseases have yet to be accomplished.

**REFERENCES**


Objective: After reading this article, participants should be able to demonstrate an increased understanding of their knowledge of allergy, asthma, and immunology clinical treatment and how this new information can be applied to their own practices.

Participants: This program is designed for physicians who are involved in providing patient care and who wish to advance their current knowledge in the field of allergy, asthma, and immunology.

Credits: ACAAI designates each Annals CME Review Article for a maximum of 2 category 1 credits toward the AMA Physician’s Recognition Award. Each physician should claim only those credits that he/she actually spent in the activity. The American College of Allergy, Asthma and Immunology is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medication education for physicians.
CME Examination

Self-Assessment Exam Questions
1. The complement system is:
   a. a group of proteins that is involved with vasculitis and joint destruction
   b. approximately 31 proteins, some of which are enzymes and other inhibitors, enzyme stabilizers, and membrane-integrated proteins involved in the innate immune system
   c. composed of too many components to understand
   d. important in the coagulation cascade

2. Complement determinations and localization:
   a. are measurable in the blood, joint fluids, and pleural and pericardial fluids
   b. can be found in skin and kidney biopsy specimens by immunofluorescence techniques
   c. neither a nor b
   d. both a and b

3. Complement-derived anaphylatoxins C3a and C5a:
   a. may be involved in immediate skin and lung pathophysiology (asthma and urticaria)
   b. provide the only chemotactic signals for neutrophils
   c. cannot be measured in blood samples
   d. are elevated in all systemic lupus blood samples

4. Hereditary angioedema is:
   a. a recently described disease associated with urticaria and tongue angioedema
   b. not associated with complement or coagulation activation
   c. a hereditary dominant condition caused by a structural protein mutation in the C1 inhibitor gene or insufficient production of C1 inhibitor protein
   d. a disease treatable with epinephrine or inhaled bronchodilators

5. Acquired diseases associated with lowered CH50 values include:
   a. systemic lupus
   b. membranoproliferative glomerulonephritis
   c. gout
   d. a, b, and c
   e. a and c
   f. a and b

Answers found on page 605.