

Clinical and laboratory evaluation of complement deficiency

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The complement system provides innate defense against microbial pathogens and is a “complement” to humoral (antibody-mediated) immunity. Consisting of plasma and membrane proteins, this proinflammatory system works in part by a cascade involving limited proteolysis whereby one component activates the next, resulting in a dramatic amplification. The overall goal is deposition of complement fragments on pathologic targets for the purposes of opsonization, lysis, and liberation of peptides that promote the inflammatory response. Deficiencies of complement components predispose to infections and autoimmune syndromes. Even though total deficiency of a complement component is rare, patients presenting with certain bacterial infections and autoimmune syndromes, especially SLE, have a much greater incidence of deficiency. This review will summarize the clinical manifestations and pathophysiology of congenital and acquired complement deficiency diseases. We will also present an algorithm for laboratory diagnosis of complement deficiency and discuss current and future therapeutic options. (*J Allergy Clin Immunol* 2004;113:585-93.)

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COMPLEMENT CASCADE FOR THE CLINICIAN

The complement system is an enzymatic reaction cascade involving 3 pathways.¹⁻³ Figs 1 and 2 depict a simplified version of the complement activation pathways and the resulting biologic consequences. Although each pathway is activated differently, they merge at the C3 step

Abbreviations used

| | |
|---------|---|
| AAE: | Acquired angioedema |
| AP: | Alternative pathway |
| C1-Inh: | C1 inhibitor |
| CP: | Classical pathway |
| HAE: | Hereditary angioedema |
| HUS: | Hemolytic uremic syndrome |
| HUVS: | Hypocomplementemic urticarial vasculitis syndrome |
| I/R: | Ischemia/reperfusion |
| LP: | Lectin pathway |
| MAC: | Membrane attack complex |
| MBL: | Mannan-binding lectin |
| PNH: | Paroxysmal nocturnal hemoglobinuria |

and have the common end points of promoting inflammation, eliminating pathogens, and enhancing the immune response. Sequentially triggered enzymatic reactions lead to the proteolytic cleavage of C3 to generate a large fragment, C3b, and a small one, C3a. Deposition of clusters of C3b on the target marks it for destruction, whereas C3a, an anaphylatoxin, triggers nearby cells to release mediators of inflammation. Subsequent cleavage of C5 results in the products C5a and C5b. C5a is also an anaphylatoxin and potent chemotactic factor. C5b initiates the membrane attack complex (MAC), which can lyse microorganisms and other target cells.^{4,5}

The 3 pathways, the classical (CP), alternative (AP), and lectin (LP), are initiated by distinct mechanisms. CP activation usually begins when C1q binds to the Fc portion of an antibody in an immune complex, although other compounds can also initiate the CP.⁶⁻⁸ Another C1 subcomponent, the serine protease C1r, then autoactivates and cleaves the serine protease C1s. C1s in turn cleaves C4 and C2. Cleavage fragments of C4 and C2 assemble on the target to form the C3 convertase (C4b2a), which in turn cleaves C3 to produce C3a and C3b. C3b deposits on the target where it serves as an opsonin and interacts with C4bC2a to form the C5 convertase. The C5b formed by the convertase initiates the terminal lytic sequence of MAC (C5-C9) by binding C6 and C7. This complex attaches to the membrane and subsequently engages C8 and multiple C9s (poly C9).⁹

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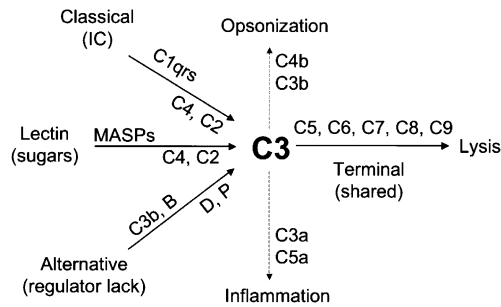


FIG 1. Schematic of complement activation pathways showing central position of C3. C3 might be activated by 3 mechanisms: (1) CP via immune complex (IC) formation, (2) LP via sugar residues, or (3) AP via amplification of the "C3 tickover" (see Figs 2 and 3) when C3 binds to surfaces that lack complement regulators.

Similar to the CP, the lectin or mannan-binding pathway is fired when lectins, synthesized in most cases by the liver, bind to a carbohydrate moiety. Mannose-binding protein (MBP), ficolin, and related lectins bind sugar residues on the microbial surface, and the associated proteases (MASPs), analogous to C1r and C1s, cleave C4 and C2.¹⁰ From that point, C4b and C2a form the CP C3 convertase, and activation proceeds to the terminal components.

Unlike the CP or LP, the initiation of the AP does not require an antibody or a lectin. This pathway is continuously turning over on a small scale ("C3 tickover").¹¹ Attachment of active C3 to a surface lacking complement regulators permits this pathway to rapidly amplify via a feedback loop, shown schematically in Fig 2. The C3b generated forms more convertases and thus gives rise to more C3b. The C3 convertase is initiated when the proenzyme, factor B, attaches to target-bound C3b. Factor B then undergoes cleavage by factor D to produce the alternative pathway C3 convertase (C3bBb). Properdin stabilizes this complex. As more C3 is cleaved by the convertase to C3b, an amplification loop is set up that permits large amounts of C3b to be deposited on the target. If another C3b binds to the AP C3 convertase, the C5 convertase is formed. The cleavage of C5 begins the assembly of the MAC, a process common to all 3 pathways.

Because of the complement system's proinflammatory and potentially deleterious effects to host cells, complement activation is very tightly controlled. Passive control of complement activation occurs because the half-life of the sites on C4b and C3b that allows them to bind to nearby surfaces and molecules is very short, and the multimolecular complexes that form the complement enzymes (C4b2a, C3bBb) are inherently unstable. Active control occurs through the action of the complement inhibitors and inactivators. The fact that nearly half of complement system proteins are engaged in regulation attests to the importance of strict control. Regulators operate at 3 stages: blocking the initiation of the cascade, preventing amplification of the C3 and C5 convertases, and inhibiting the terminal MAC. C1 inhibitor (C1-Inh) acts in the early steps and prevents chronic and excessive

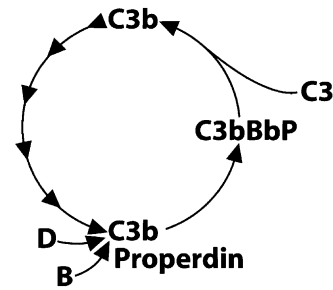


FIG 2. The AP amplification loop allows continuous production of C3b. The cleavage of C3 is accelerated when active C3b binds to microbial or tissue surfaces that lack natural complement regulators, setting in motion an efficient biological amplification process.

complement activation of the CP and LP by blocking active sites of C1r, C1s, and the MASPs.¹² The C3 convertases are the main amplifiers. Regulation of the convertases is accomplished by destabilizing enzymatic complexes and by proteolytic degradation of active fragments by factor I (C3b-inactivator). The inhibitors include membrane-bound proteins (decay accelerating factor, complement receptor 1, membrane cofactor protein) and plasma proteins (C4b-binding protein and factor H).¹³ Finally, CD59 on cells, Protein S (vitronectin), and possibly SP-40,40 (clusterin) in plasma block assembly or insertion of the MAC on the cell membrane.

Deficiency of any one of these regulator proteins results in excessive complement consumption, leading to an inappropriate inflammatory response, destruction of self-tissue, and depletion of C3 or other components downstream of the missing control protein.

COMPLEMENT DEFICIENCY DISEASES

Genetics

Most complement components are inherited in an autosomal codominant pattern. Typical components of this type include C1-Inh, C2, C3, C5, C6, C7, and C9. The gene defects range from single nucleotide changes that might result in a dysfunctional protein to complete deletion with no protein produced. Parents of patients with complete deficiency of a complement component generally have concentrations of that component that are less than the mean of the normal range, indicative of the heterozygous condition. Although complete deficiencies have been described for most of the known complement components, C1-Inh deficiency has presented only as the heterozygous state. No patient with inherited total factor B deficiency has been confirmed to date. Properdin deficiency is the only complement deficiency that is X-linked, so all the known cases are male.

The genetics of C1, C4, and C8 deficiency are more complex, involving multiple genes for each component. C1q, C1r, and C1s are required for C1 function; thus if any one subunit is missing, the complex cannot form. C4 exists in humans in 2 forms, C4A and C4B, both of which are coded for by different genes and are present in equal amounts in approximately 75% of the normal

population.¹⁴ The C4 genes (also Factor B and C2) are present in the MHC on chromosome 6. The few amino acid differences between the 2 forms of C4 affect only their ability to bind to proteins (C4A) or carbohydrate residues on cell surfaces (C4B), so standard assays for C4 do not distinguish between the forms. C4A deficiency (lacking both C4A genes) is increased (8% to 12%) in white patients with lupus compared to control subjects (1% to 3%). C8 is made up of 3 chains that are encoded for by different genes. Because C8 requires all 3 chains to be functional in the MAC, assays that measure only the protein can be misleading, whereas the functional assay is diagnostic.

Incidence

Total inherited deficiency of a complement activating protein is rare in the general population. The most convincing demonstration of the scarcity of a complete complement deficiency in healthy patients came from a study of 145,640 Japanese blood donors.^{15,16} With the exception of 139 patients who were found to have isolated C9 deficiency (which is increased in this population), no patient had CP or AP complement component deficiency. Complement deficiency also was not found in a population survey of more than 4000 Swiss army recruits.¹⁷ It is estimated that the prevalence of an inherited complete complement deficiency is 0.03% in the general population, excluding deficiency of mannan-binding lectin (MBL), which might be present in the homozygous form in as many as 3% of people.¹⁸ However, the incidence of complement deficiencies is much higher in populations that have specific disease entities. For example, in white patients with rheumatologic disorders such as SLE, the incidence of C2 deficiency approaches 1% (as opposed to the general population, in whom incidence is estimated to be 0.009% to 0.01%).^{19,20} Complement deficiency also predisposes patients to infection. In patients with recurrent disseminated neisserial infections, the incidence of complement deficiency has been estimated to be as high as 20%.²¹

Congenital complement deficiency diseases

As long as innate immunity is preserved, the absence of an early CP component (C1, C4, or C2) usually does not predispose overwhelmingly to severe infections observed in patients with deficiency of properdin, C3, or a terminal component. However, it is not uncommon for patients with C2 deficiency to present with milder recurrent bacterial infections. Somewhat paradoxically, deficiencies of early CP components are commonly linked to autoimmune disorders, particularly SLE. Table I provides a list of the presenting syndromes and the component(s) likely to be associated with the clinical condition.

Infections

Patients with a complement component deficiency often present with pyogenic infections, particularly with encapsulated bacteria such as *Streptococcus pneumoniae* and *Haemophilus influenzae type b*. Those with deficiency

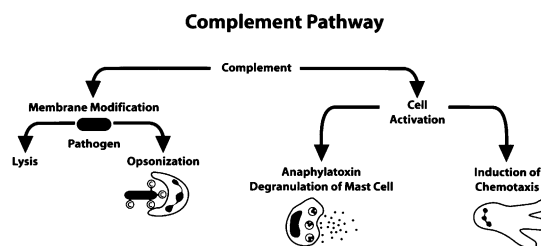


FIG 3. Biological effects of complement activation.

of the early components of the CP (C1, C4, or C2) might also present with these same infections (especially at an early age), but more commonly as teens and young adults they develop autoimmune syndromes.²²⁻²⁴ Deficiency of C3, the major opsonin, results in severe, recurrent pyogenic infections that begin shortly after birth, a clinical presentation and course similar to that observed in hypogammaglobulinemia.²⁵⁻²⁷ Acquired C3 deficiency, such as with factor H or factor I deficiencies or the presence of C3 nephritic factor, predisposes the patient to the same risks.

A second type of infectious disease presentation in patients with complement deficiency is recurrent infection by *Neisseria gonorrhoeae* or *N meningitidis*. This occurs in the setting of a deficiency of a MAC component (C5, C6, C7, or C8)^{21,25} or of the AP component properdin.²⁸ Deficiency of MBL has also been linked to an increased frequency of pyogenic infections and sepsis, particularly in children and neonates.^{29,30} Last, leukocyte adhesion deficiency syndrome is a result of the lack of complement receptor 3, an integrin that binds degradation products of C3b.^{31,32} First suspected at birth because of delayed umbilical cord separation, this disease usually results in death during childhood as a result of refractory infections involving soft tissues and mucosal surfaces.³⁰

Rheumatic and connective tissue disease

The most common presentation of an individual with an early component deficiency (C1, C4, or C2) is an autoimmune disease. Although a wide variety of such syndromes have been associated with CP deficiency, the strongest association is with SLE. The incidence of SLE in patients with C1q, C4, or C2 deficiency is 90%, 75%, and ~15%, respectively.¹⁷ Partial C4 deficiency is also associated with SLE; 15% of patients with SLE exhibit C4A deficiency. SLE in patients with complement deficiencies has characteristic features including earlier age of onset, prominent photosensitivity, lower frequency of renal disease, and variable antinuclear antibody titers (although anti-Ro is present in two thirds of patients). Also, there is a nearly equal male to female ratio in the case of SLE presentation with C1q or C4 deficiency.^{26,33}

There is also a 2- to 3-fold increase in MBL deficiency in patients with lupus.³⁴ These individuals tend to have more frequent and severe infections in the course of their rheumatic disease. Worse outcomes are also observed in patients with cystic fibrosis or rheumatoid arthritis associated with MBL deficiency.³⁵

TABLE I. Inherited complement deficiencies and clinical associations

| Presenting syndrome | Component | Pathway | Inheritance | Major clinical correlates |
|---------------------|---------------------------------|------------------------------|----------------------------------|---|
| Infection | C1q, C1r, C1s, C4, C2 | CP | Autosomal | Most commonly autoimmune conditions, particularly SLE. Also higher than normal incidence of encapsulated bacterial infections. |
| | C3 | Common to CP, AP, LP | Autosomal | Severe, recurrent pyogenic infections early in life. Also might have glomerulonephritis. |
| | C5, C6, C6, C8, or C9 | MAC | Autosomal | Recurrent <i>Neisseria</i> infections, less common with C9. |
| | Factor H, factor I | AP inhibitors | Autosomal | Recurrent pyogenic infections as a result of C3 deficiency. Factor H deficiency is also associated with glomerulonephritis and HUS. |
| | Properdin MBL | AP stabilizer LP | X-linked Autosomal | Recurrent <i>Neisseria</i> infections Pyogenic infections and sepsis in children and neonates; also an association with SLE |
| | CR3 | Receptor | Autosomal | Leukocyte adhesion defect: leukocytosis, pyogenic infections, delayed umbilical cord separation |
| Rheumatic disorders | C1, C2, C4 | CP | Autosomal | As above; mainly SLE |
| HAE | MBL | LP | Autosomal | As above; SLE is associated as well. |
| Kidney damage | C1-Inh | CP inhibitor | Autosomal | HAE |
| | C3 | Common to CP, AP, LP | Autosomal | Membranoproliferative glomerulonephritis and overwhelming infections |
| | Factor H CD46 | AP inhibitor AP inhibitor | Autosomal Autosomal | Atypical HUS, glomerulonephritis Atypical HUS |
| PNH | Decay accelerating factor, CD59 | AP and MAC inhibitors | Somatic mutation on X chromosome | Hemolysis and thrombosis |

Hereditary angioedema

Hereditary angioedema (HAE) is characterized by recurrent episodes of nonpainful, nonpruritic, and non-erythematous subcutaneous and submucosal swelling that spontaneously subsides in 48 to 72 hours.^{12,36,37} It is not accompanied by urticaria and is not responsive to antihistamines, steroids, or β -agonists.³⁶ Symptoms usually begin in adolescence and range in severity from a cosmetic inconvenience (facial, truncal, and extremity edema) to life-threatening laryngeal edema. Edema of the bowel wall is common and results in severe colicky abdominal pain, nausea, and vomiting. HAE is distinguished from acute abdominal syndromes by the absence of fever, peritoneal signs, and an elevated white blood cell count.^{34,35} Swelling usually arises sporadically and spontaneously, but in up to one half of the cases it might be triggered by mild trauma or possibly psychological stress. Angiotensin inhibitors increase attack frequency.³⁶

HAE results from heterozygous deficiency of C1-Inh.³⁷ The C1-Inh is an important regulator not only of C1r and C1s but also of Hageman factor, clotting factor XI, plasma kallikrein, and plasmin. The biochemical mediator(s) responsible for angioedema could arise therefore from the coagulation, complement, or kinin-generating pathways. It has not been definitively identified. More than 100 mutations in the C1-Inh gene have been reported in

unrelated patients, and approximately 20% of patients represent new mutations (no family history).³⁸⁻⁴³

Even though they behave the same clinically, there are 2 biochemically characterized forms of HAE. Comprising 85% of kindreds, Type I HAE is defined by a reduction of C1-Inh to approximately 30% or less of normal antigenic and functional levels. In Type II, on the other hand, patients have normal or elevated antigenic levels but synthesize a dysfunctional protein with reduced or absent C1-Inh function. One recent study identified a third type in which the patients, all of whom were women, had clinical findings consistent with HAE but normal C1-Inh level and function.⁴⁴ The underlying defect has not been identified.

Kidney damage

Development of kidney disease in the presence of autoimmunity occurs by several mechanisms. First, immune complexes formed in blood can become lodged in the kidney and provoke damage (SLE, serum sickness, mixed cryoglobulinemia). Second, antigens in the form of DNA or other components associated with the glomerular basement membrane can attract circulating antibodies such that immune complexes form de novo in the kidney. Third, autoantibodies in conditions like Goodpasture's disease bind to collagen in the glomerular basement

membrane and then activate complement to incite inflammation.

Reduced C4 and C3 levels in the setting of lupus nephritis are an important predictor of more severe disease and poor outcome.^{17,45} Total deficiency of C3 is associated with development of membranoproliferative glomerulonephritis.⁴⁶ Recently, a link between complement deficiency and atypical, nondiarrheal-associated hemolytic uremic syndrome (HUS) was identified; heterozygous mutations in factor H, a plasma complement regulator, were found in several cohorts of HUS patients.⁴⁷ Complete factor H mutations are associated with membranoproliferative glomerulonephritis in several animal models and in humans.⁴⁸ Recently, 2 groups identified heterozygous mutations in CD46, a widely expressed transmembrane complement regulator, in patients with atypical familial HUS.^{49,50} The underlying message is that when regulators are missing, the system amplifies excessively, often to exhaustion, and, in so doing, damages self-tissue. The kidney appears to be particularly vulnerable to this type of injury.

Acquired complement deficiency diseases

Autoimmune diseases, especially those featuring immune complexes, often result in secondary complement deficiency because complement activation outstrips hepatic synthesis. These partial deficiencies usually abate with disease management and therefore can be used as a guide to disease activity. Other conditions leading to depletion of circulating complement include autoantibodies to complement components such as C3 and C4 nephritic factors that bind to and stabilize the C3 convertases of the AP and CP, the antibody to C1-Inh mentioned earlier, and antibody to the collagen-like region of C1q that is associated with hypocomplementemic urticarial vasculitis syndrome (HUVS) and is also present in about 30% of SLE patients. Transient activation and depletion of complement can also occur with sepsis, viremia, burns and other trauma, ischemic injury of many organs, and some drug reactions.

Immune complex deposition

Complement promotes the clearance of foreign antigens through immune complex formation. C1q, C4b, and C3b coat the immune complex, maintain its solubility (ie, keep it from precipitating), and provide ligands for the complex to attach to cells (the immune adherence reaction). If a large complex forms because the CP is compromised as a result of deficiency or depletion of 1 or more components, activation of the AP can lead to deposition of C3b through the amplification loop. Another factor that plays an important role in the clearance of immune complexes is the binding to C3b receptors, such as complement receptor 1 on erythrocytes, that then carry the complexes to the liver and spleen where they are eliminated by the resident macrophages. Depletion of complement receptor 1 by this process can occur in patients with immune complex disease and contribute to increased difficulty clearing more complexes.

Immune complexes that are not properly eliminated can cause inflammation. In lupus, autoantibodies are generated against nuclear antigens, and, with cell breakdown, they form excessive amounts of immune complexes that inappropriately deposit in vascular structures.⁵¹ Serum sickness is caused by the presence of a relatively large amount of foreign antigen (usually a protein) to which the host mounts an immune response.⁵² Mixed cryoglobulinemia is often caused by the immune response to hepatitis C virus or by complexes with rheumatoid factor. Patients with cryoglobulinemia form complement-fixing immune complexes and thereby incite inflammatory responses in their joints and nerves.⁵³

Autoantibody syndrome

In the Type II hypersensitivity reactions, the autoantibody binds to a fixed antigen on cells or a tissue site and activates complement. In myasthenia gravis, for example, the autoantibody binds to the acetylcholine receptor and then fixes complement.⁵⁴ This combination of immune reactants triggers reactions that damage the receptor. Goodpasture's syndrome is another well-studied example of this general paradigm. Acquired angioedema (AAE) is caused by clearance of C1-Inh, either through excessive activation of C1 or other enzymes that react with it or by autoantibodies to the C1-Inh.⁵⁵⁻⁵⁷ Even though its clinical features are indistinguishable from those of HAE, factors such as lack of family history, later age of onset, and the presence of a malignancy (in ~50%) favor AAE over HAE.^{40,58}

C3 nephritic factor is an autoantibody against the AP pathway convertase. It results in secondary C3 deficiency that can be almost complete because the overly stabilized AP fires to exhaustion. Children are most often affected and present with the triad of membranoproliferative glomerulonephritis, partial lipodystrophy, and frequent bacterial infections.⁵⁹ Autoantibody to the collagen-like region of C1q also occurs in SLE patients with renal symptoms and is also present in all patients with HUVS.^{60,61}

Paroxysmal nocturnal hemoglobinuria

A rare acquired disorder of stem cells, paroxysmal nocturnal hemoglobinuria (PNH) is characterized by intravascular hemolysis with hemoglobinuria and venous thrombosis of major vessels.⁶² PNH erythrocytes are highly susceptible to complement-mediated lysis because of the absence of 2 complement regulators, decay accelerating factor (CD55) and inhibitor of the MAC (CD59). Both of these regulators are tethered to the cell membrane by a glycosylphosphatidylinositol anchor. The acquired molecular defect in PNH is a mutation in the *Pig-A* gene on the X chromosome whose protein product is necessary to synthesize the glycosylphosphatidylinositol anchor.⁶³

Ischemia-reperfusion injury

Reperfusion of ischemic tissue is associated with a marked inflammatory reaction that might lead to further

undesirable tissue and organ damage. Much evidence points to complement activation being involved in ischemia-reperfusion (I/R) injury. Animal models suggest that salvage of a substantial amount (10% to 40%) of the ischemic tissue can be achieved by blocking complement on reperfusion.⁶⁴ Depleting C3 by cobra venom, for example, reduces I/R damage to the kidney and the heart, and recombinant soluble complement receptor 1 (which blocks the activity of C3 and C5 convertases) is effective in models of myocardial infarction, lung/liver transplantation, intestinal I/R, and stroke.⁶⁵⁻⁶⁷ The major mediators of damage are believed to be the anaphylatoxins, C3a and C5a, that attract neutrophils that in turn release enzymes and other mediators and also generate oxygen radicals.

Apoptosis

The relationship between complement and apoptosis, a form of programmed cell death, is just beginning to be explored. Complement-mediated cell lysis was thought to result in only necrotic cell death until the MAC was demonstrated to cause apoptotic cell death in tissues after I/R.^{68,69} Sublytic doses of the MAC induce cell stimulatory effects, including enhanced cell protection from apoptosis.⁷⁰ Complement has also been implicated in a "waste disposal" role relative to apoptotic cells.² C1q binds to cells undergoing apoptosis and facilitates elimination of such cells. If the complement system does not succeed at this disposal of garbage, the partially degraded and damaged components of the cell could accumulate, be modified, and then evoke autoimmune responses. This mechanism is proposed as a "garbage" hypothesis to explain SLE.² At this juncture, examination of the role of complement in mediating apoptosis and clearing apoptotic cells is still in its infancy.

DIAGNOSTIC TESTS FOR COMPLEMENT DEFICIENCY

Functional screening tests

Laboratory tests for complement components include tests for functional activity of the CP (CH50 and its equivalents), the AP (AH50 or APH50), and the MBL pathway. The CH50 is based on a hemolytic assay in which an immune complex is formed by adding antibodies that react with a surface antigen on sheep red blood cells.⁷¹ When complement is activated by the antigen-fixed antibodies on the cell surface, the cell is lysed and hemoglobin is released. Because the formation of the MAC on the cell requires the sequential action of all 9 components of the classical (C1, C4, and C2) and terminal (C3, C5, C6, C7, C8, and C9) pathways, by titrating the complement source (serum in most cases) so that only a portion of the cells present are lysed, the amount of active complement can be calculated, and the results are expressed as the reciprocal of the dilution of serum that caused lysis of 50% of the cells in the assay.

Two variants of the CH50 are currently in use in clinical laboratories: an assay based on lysis of liposomes that

release an enzyme that can be read on an analyzer of the sort used for other clinical laboratory tests (WAKO Clinical Diagnostic Reagents, Richmond, Va) and solid phase assays (ELISAs) that detect the final C9 neoantigen that is formed when the complete pathway has been activated (Quidel, San Diego, Calif; Diasorin, Stillwater, Minn). The CH50 is the best single screen for complement abnormalities in that absence or decrease of activity in the CH50 implies that at least one of the necessary components is missing or low.⁷²

The analogous assay for AP activity, the AH50, is not as widely available as the CH50, but it is useful as a screen for complement deficiency especially when used in conjunction with the CH50. The AH50 depends on the unique properties of erythrocytes from certain species to provide a surface that promotes activation of the AP, with sequential activation of factors D, B, P, C3, C5, C6, C7, C8, and C9.⁷³ Properdin is required for stabilization of the C3 convertase (C3bBb), and inefficient activation of the AH50 occurs if P is low or absent. The most common variation of the AH50 uses rabbit red cells in combination with a buffer that blocks activation of the CP or LP. Like the CH50, the AH50 is a measure of the percentage of rabbit erythrocytes lysed by diluted serum and is expressed in units that represent the dilution that lyses 50% of the cells used in the assay.

If both CH50 and AH50 are used to screen for complement deficiency, the number of additional tests required to pinpoint the defect can be minimized (Fig 4).⁷² Because both assays include the same 6 terminal components (C3, C5, C6, C7, C8, and C9), the results will be low or absent for both tests if 1 or more of these components is missing. If a CP component is missing, the CH50 will be low or absent, but the AH50 will be normal, whereas if an AP component is low or missing, the reverse will be true. Table II provides a generalized guide to choosing tests for differentiating between acquired and inherited complement deficiencies.

Several assays for C1-Inh function have been developed. One that is commonly used is an ELISA that measures complexes formed between biotinylated C1s and C1-Inh that are captured on an avidin-coated plate (Quidel). A drawback of this assay is that the rare patient might have normal function in the assay but a mutation of the C1-Inh that allows binding of C1s but not of 1 or more of the other enzymes with which the inhibitor reacts.

Another test for C1-Inh function relies on the observation that binding of the inhibitor to C1r masks the site on C1r that is recognized by some polyclonal antibodies.⁷⁴ After activation of the test serum with aggregated immunoglobulins, the amount of C1r detectable by radial immunodiffusion decreases in proportion to the amount of active C1-Inh present. Functional tests can be done for each of the individual components by using variations on the CH50 or AH50 assays in which an excess of all components except the one being evaluated is added to the appropriate cells, and the patient's serum provides the only source of the component in question.⁷⁵

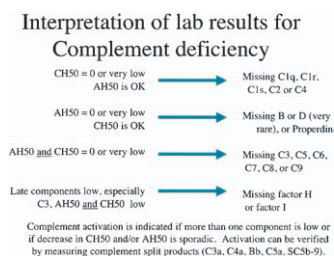


FIG 4. Interpretation of laboratory results and follow-up tests after CH50 and AH50.

Alternatively, purified components can be added to the patient's serum to determine which one(s) restore activity.

The function of the MBL pathway can be determined by using an ELISA in which the patient's serum is placed into wells coated with mannan.⁷⁶ After MBL binds to the mannan-coated surface, the MASP enzymes cleave C4, and the resulting C4b and C4d that are deposited on the plate can be measured by using enzyme-conjugated mAbs.

Quantitative tests for component concentrations

Like most other circulating proteins, the complement components can be measured by immunochemical methods common in most laboratories. These include immunoprecipitation assays including nephelometry, radial immunodiffusion, RIA, and ELISA techniques. The critical points are the specificity of the antibodies used and the reliability of the standard and controls. There are as yet very few complement assays that have been standardized and validated for Food and Drug Administration approval, and most laboratories must rely on in-house methods and proven research technology to perform diagnostic procedures for complement analysis.

The most definitive method for evaluating complement activation is in quantitation of the fragments formed during the enzymatic cleavage steps.⁷⁷ Because many of the complement components are acute phase reactants, decreases due to activation might be masked by increases in the synthesis rates during an inflammatory episode. The split products can be used to determine whether activation has occurred, because their increase occurs only when the complement enzymes are formed and active. An added bonus is that the pathway of activation can be determined; C4a and C4d are markers for CP or LP activation, Bb is a marker for AP activation, and C3a, iC3b, C5a, and soluble C5b-9 can be used to determine terminal pathway activation.

Complement autoantibody tests

C1q and C1-Inh autoantibodies can be determined by using ELISA methods.⁷⁸ The antibodies to C1-Inh bind to the inhibitor molecule and prevent it from attaching to the enzyme, but they do not prevent the enzyme from cleaving the inhibitor. The resulting lower molecular weight inhibitor fragment can be detected by PAGE.⁷⁹ There are several assays available for C3 nephritic factor that rely

TABLE II. Distinguishing hereditary from acquired deficiencies

| Acquired vs hereditary complement deficiencies | | |
|---|-------------------------|---------------------------------|
| Test result | Acquired | Hereditary |
| CH50 and/or AH50 | Low | Absent |
| Component levels or function | Multiple components low | Only 1 component low or absent* |
| Family history | None | Consistent with deficiency |

*The exception to this rule is when a control protein (C1-INH, H, I) is missing.

on its function and either measure lysis due to generation of C3b on a red cell surface or look directly at the amount of C3 that is cleaved when the patient's serum is mixed with normal serum.⁸⁰

COMPLEMENT THERAPEUTICS IN CLINICAL PRACTICE

Treatment of patients with congenital complement deficiencies focuses on the underlying problems of infection and autoimmunity. Recombinant complement components for a completely deficient patient are possible, but economic considerations are such that such purified proteins are unlikely to become available. Blood transfusion to replace missing components has been tried with some success in 2 SLE patients with C2 deficiency and several patients with factor H deficiency.^{33,48} Replacement of the C1-Inh, especially at the time of attacks, has been successful. Renal transplantation might be a viable therapy specifically for atypical HUS patients with an MCP mutation.

On the other hand, much effort has gone into using recombinantly produced complement inhibitors in I/R injury and autoimmune disorders. Several inhibitors have been tested in animal models, most notably sCR1, which is active against the C3 and C5 convertases of both the AP and CP,⁸¹⁻⁸³ and a humanized anti-C5 mAb, a recombinant antibody against C5, that prevents cleavage of C5 to C5a and C5b.⁸⁴⁻⁸⁷ Numerous studies in animal models have demonstrated the efficacy of both inhibitors in preventing a variety of I/R injuries, especially in murine models. In addition, these 2 inhibitors were efficacious in delaying the progression in models of rheumatoid arthritis and SLE in mice.^{85,86} The success of these animal studies led to clinical trials, with sCR1 being used for treatment of acute respiratory distress syndrome, myocardial infarction, lung transplantation, and post-cardiopulmonary bypass syndrome and anti-C5 mAb in multicentered trials for myocardial infarction, post-cardiopulmonary bypass syndrome, rheumatoid arthritis, membranous nephropathy, and lupus nephritis.⁸⁸ The results of these trials have only been partially published, but in general the effects have been positive but modest. In PNH, however, complement inhibition led to a prompt reversal of the

hemolytic process.⁸⁹ We are still awaiting results from many of the multicentered trials.

SUMMARY

As part of innate immunity, the complement system provides an important effector arm for many of its functions, including host defense, regulation of acquired immunity, and clearance of immune complexes and other potentially dangerous material. Complement deficiencies have been documented for almost all of the known components of the complement system including the cell-associated receptors and control proteins. The resulting diseases in these patients include recurrent mild to severe, life-threatening infections, increased incidence of auto-immune disorders, and recurrent edema, urticaria, and vasculitic syndromes. Studies in animal models, particularly knockout or transgenic mice, have confirmed many of the earlier studies showing the importance of the complement system in maintaining health. It is hoped that targeted gene therapy or replacement with recombinant proteins will be available to help patients with deficiencies of complement in the future.

Additional studies are ongoing to expand our knowledge of complement to the point at which its activation can be blocked by specific compounds including mAbs and new inhibitors to prevent many of the sequelae of autoimmune or I/R injury. On the other hand, the power of the complement system might be exploited to destroy specific target tissues such as tumors by directing activation to the cells to be destroyed.

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