Complement, as a vital part of the body’s immune system, provides a highly effective means for the destruction of invading microorganisms and for immune complex elimination (for reviews, see references 77 and 78). Through activation and interaction with its respective receptors on various immune cells, complement is closely linked to the adaptive immune response. A normally functioning complement system is also required for physiological tissue regeneration and repair (37). Thus, complement defects (Table 1) increase the susceptibility to infection and, if classical pathway components are affected, are frequently associated with autoimmune disorders (11). Protection against complement-mediated tissue destruction is provided by various soluble and membrane-bound regulatory proteins (Fig. 1) (51). Defects in this regulation may therefore lead to serious disorders due to unrestricted activation. In addition, as a key mediator of inflammation, complement also significantly contributes to tissue damage in various clinical disorders (50, 76).

The present paper briefly reviews clinical indications for complement analysis and gives an overview on current methods in routine and experimental complement diagnostics.

**THE COMPLEMENT SYSTEM**

Complement is activated by three pathways (Fig. 1), the classical pathway, the alternative pathway, and the recently discovered lectin pathway (14), all of which lead to the formation of the cytolytic membrane attack complex, C5b-9. Following complement activation, the biologically active peptides C5α and C3a elicit a number of proinflammatory effects, such as chemotaxis of leukocytes, degranulation of phagocytic cells, mast cells, and basophils, smooth muscle contraction, and increase of vascular permeability (29). Upon activation by these complement products, the inflammatory response is further amplified by subsequent generation of toxic oxygen radicals, induction of synthesis, and release of arachidonic acid metabolites and cytokines. Consequently, an (over)activated complement system presents a considerable risk of harming the host by directly and indirectly mediating inflammatory tissue destruction.

Under physiological conditions, activation of complement is effectively controlled by the coordinated action of soluble and membrane-associated regulatory proteins (51). Soluble complement regulators, such as C1 inhibitor, C4b-binding protein (C4bp), factors H and I, clusterin, and S-protein (vitronectin), restrict the action of complement in body fluids at multiple sites of the cascade reaction (Fig. 1). In addition, each individual cell is protected against the attack of homologous complement by surface proteins, such as the complement receptor 1 (CD35) and the membrane cofactor protein (CD46), as well as by the glycosylphosphatidylinositol-anchored proteins, decay-accelerating factor (DAF; CD55), and CD59. There is increasing evidence that these protection mechanisms are augmented on malignant cells, leading to their refractoriness against complement (26).

**CLINICAL INDICATIONS FOR COMPLEMENT ANALYSIS**

Clinical and experimental evidence underlines the prominent role of complement in immunodeficiency disorders (Table 1) and in the pathogenesis of numerous inflammatory diseases (11, 50, 76), including immune complex and autoimmune disorders such as systemic lupus erythematosus (SLE) (79) and autoimmune arthritis (34). The following are disorders associated with complement activation: systemic inflammatory reaction syndrome, multiple organ dysfunction syndrome, ischemia-reperfusion syndrome, angioedema, capillary leak syndrome, hyperacutie and acute graft rejection, vasculitis, nephritis, autoimmune disorders (e.g., SLE, rheumatoid arthritis, and myasthenia gravis), biomaterial incompatibility (e.g., following dialysis or cardiopulmonary bypass), and severe trauma, burn, and sepsis. Only recently has complement also been implicated in neurodegenerative disorders, such as Alzheimer’s disease (52), multiple sclerosis (70), and Guillain-Barré syndrome (62). Complement activation after polytrauma substantially contributes to the development of systemic inflammatory reaction syndrome and multiple organ failure (82). In recent years, complement has been recognized as a major effector mechanism of reperfusion injury (35, 46, 48). Furthermore, activation of complement is a critical event in the pathogenesis of sepsis and septic shock (10, 15).

The inflammatory response induced by artificial surfaces in hemodialysis and extracorporeal circuits may lead to organ dysfunction. Here, complement activation has been shown to be associated with transient neutropenia, pulmonary vascular leukostasis, and occasionally, anaphylactic shock of variable severity in patients undergoing hemodialysis or cardiopulmonary bypass (27, 47).

In recent years, great progress has been made in complement analysis to better define disease severity, evolution, and response to therapy. Modern diagnostic technologies which focus on the quantification of complement-derived split products or protein-protein complexes now provide a comprehensive insight into the activation state of the system.

(i) **Recurrent infections.** Complement defects (Table 1) may be the cause of immunodeficiency-mediated recurrent infec-
<table>
<thead>
<tr>
<th>Component</th>
<th>Disease condition(s)</th>
<th>Analytical method(s)</th>
<th>Genetic basis(es)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1q, C1s</td>
<td>Collagen vascular disease, SLE, bacterial infections</td>
<td>CH50, RID, ELISA, C1q/C1s function, SDS-PAGE</td>
<td>Various point mutations; acquired form due to C1q autoantibodies</td>
</tr>
<tr>
<td>MBL</td>
<td>Bacterial infections</td>
<td>MBL function, ELISA</td>
<td>Various point mutations</td>
</tr>
<tr>
<td>C2</td>
<td>Neisserial infections, respiratory infections, SLE (often symptomless)</td>
<td>CH50, RID, ELISA, C2 function, SDS-PAGE</td>
<td>Type I, 28-bp deletion at exon-intron junction 6 leading to splicing error; linked to HLA-A25-B18-C2Q0-C4A4,B2-DR2; Type II, impaired C2 secretion</td>
</tr>
<tr>
<td>C3</td>
<td>Bacterial infections, glomerulonephritis</td>
<td>CH50, RID, ELISA, C3 function, electrophoresis</td>
<td>Various independent mutations in the C3 gene; 800-bp deletion due to Alu repeat-induced mutation; acquired due to C3 nephritic factor</td>
</tr>
<tr>
<td>C4A/B</td>
<td>Collagen vascular disease, autoimmune diseases (e.g., SLE, autoimmunehepatitis, scleroderma)</td>
<td>CH50, RID, ELISA, SDS-PAGE, electrophoresis with hemolytic overlay</td>
<td>1. Complete gene deletion; 2. gene conversion (isotype change); 3. nonexpression due to 2-bp insertion 3 stop codon</td>
</tr>
<tr>
<td>C5</td>
<td>Recurrent neisserial infections</td>
<td>CH50, RID, ELISA, C5 function</td>
<td>Point mutation 3 stop codon</td>
</tr>
<tr>
<td>C6</td>
<td>Recurrent neisserial infections</td>
<td>CH50, RID, ELISA, C6 function</td>
<td>Partial deficiency: splice site mutation 3 loss of exons 16 and 17 (3/H11032 truncation)</td>
</tr>
<tr>
<td>C7</td>
<td>Recurrent neisserial infections</td>
<td>CH50, RID, ELISA, C7 function</td>
<td>Various independent point mutations and 1-bp deletions, substitutions, insertions, deletions, frameshifts, amino acid substitutions, point mutations, 3 stop codons, 310-bp insertion and premature stop codon</td>
</tr>
<tr>
<td>C8 (C8A/H9251 – C8B/H9252)</td>
<td>Recurrent neisserial infections, C8 function</td>
<td>CH50, ELISA, SDS-PAGE</td>
<td>C8B gene: point mutation in exon 9 3 stop codon (common), also other rare mutations; C8A gene: splice site mutation in intron 6 10-bp insertion and premature stop codon</td>
</tr>
<tr>
<td>C9</td>
<td>Recurrent neisserial infections, SLE (often symptomless)</td>
<td>RID, ELISA, C9 function</td>
<td>Point mutations 3 stop codon; cysteine substitution</td>
</tr>
<tr>
<td>PF</td>
<td>Fulminant neisserial infections, sepsis</td>
<td>AH50, ELISA</td>
<td>Various point mutations 3 stop codons, frameshifts, amino acid substitution</td>
</tr>
<tr>
<td>Factor B</td>
<td>Only heterozygotes detected, clinically inapparent</td>
<td>AH50, RID, ELISA, SDS-PAGE</td>
<td>Unknown</td>
</tr>
<tr>
<td>Factor H</td>
<td>Neisserial infections, HUS</td>
<td>RID, ELISA (reduced C3 levels), SDS-PAGE</td>
<td>4-bp deletion 3 stop codon, point mutations 3 stop codon, cysteine substitutions (impaired secretion), various point mutations, substitutions, insertions, deletions, frameshifts, amino acid substitutions, point mutations, 3 stop codons, 310-bp insertion and premature stop codon</td>
</tr>
<tr>
<td>Factor I</td>
<td>Meningitis, pyogenic infections</td>
<td>CH50, RID, ELISA (reduced C3 levels and no C3d detectable)</td>
<td>Evidence for Alu repeat-induced mutation</td>
</tr>
<tr>
<td>C1 inhibitor</td>
<td></td>
<td></td>
<td>Various point mutations and small deletions, exon deletions due to Alu repeat recombinations; acquired autoimmune form</td>
</tr>
<tr>
<td>DAF, CD59</td>
<td>Paroxysmal nocturnal hemoglobinuria</td>
<td>Acidic lysis test, FACS analysis</td>
<td>GPI anchor defect due to PIG-A gene point and frameshift mutations</td>
</tr>
<tr>
<td>CR3 (CD11b/CD18)</td>
<td>Recurrent bacterial skin infections</td>
<td>FACS analysis</td>
<td>Deficiency of common ( \beta ) chain</td>
</tr>
</tbody>
</table>

Table adapted from reference 68 with permission of the publisher.

CH50, complement hemolytic activity; AH50, alternative pathway hemolytic activity; RID, radial immunodiffusion; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; FACS, fluorescence-activated cell sorter.

GPI, glycosylphosphatidylinositol; PIG-A, phosphatidylinositol glycan-A (gene).
tions (11). Recurrent neisserial infections, particularly if systemic and with rare serotypes, are associated with complement defects, preferentially in the terminal C5-C9 pathway. The frequency of meningococcal disease reported in C5-C9-deficient individuals reflects a 1,000- to 10,000-fold-higher risk in this population (65). Late-component-deficient patients, which are readily identified by assays of total complement function should be vaccinated with tetravalent vaccines against all four strains of *Neisseria meningitidis* (A,C,W135, and Y) (49).

There is also a high prevalence of properdin deficiency in patients with meningococcal disease, mainly caused by uncommon serogroups W135 and Y, with a fatality rate of more than 25% (33). The most common type I deficiency is characterized by the absence of properdin in plasma, whereas in type II deficiency, properdin is low but detectable (<10% of the normal level). However, properdin defects are not always detected by the conventional hemolytic assay (AH50). Thus, direct quantification of properdin or the application of a lipopolysaccharide (LPS)-based alternative pathway activation enzyme-linked immunosorbent assay (ELISA) (see below) may be indicated in such cases.

Mannose binding lectin (MBL) is a key protein of the lectin activation pathway of complement. Genetic deficiency of MBL is rather frequent and is associated with increased infection risk (28), particularly in the mother-child window age of 6 to 18 months. MBL should therefore be added to the list of parameters to be tested if immunodeficiency is suspected. However, the interpretation of the results should be done with some caution since, due to genetic variants (18), concentrations of MBL per se do not fully reflect the activity of the lectin pathway. Here, recently developed functional assays (30, 59) should be included.

(ii) Autoimmune diseases. Complement defects, particularly of the classical pathway, are frequently associated with SLE-like autoimmune disease (79). The strength of the association of a complement deficiency with systemic lupus erythematosus increases from C2 (10% prevalence) to C1r/s (57% prevalence), C4 (75% prevalence) and C1q (90% prevalence) (61).

In general, autoimmunity is only occasionally associated with a genetic complement defect. Therefore, in active SLE, particularly with renal involvement, low CH50 and C4 titers are more often due to increased in vivo activation, which can be verified by the detection of complement activation products. In patients with severe clinical outcome, such as lupus nephritis, autoantibodies to C1q are often found and may be of prognostic value (7).

(iii) Membranoproliferative glomerulonephritis (MPGN) and hemolytic uremic syndrome (HUS). In certain vasculitides and kidney diseases, a substantial activation and consumption of C3 due to defective alternative pathway regulation can be observed.

Patients suffering from MPGN, especially of the histologically defined type II, often show low levels of CH50, AH50, and C3. This results from a continuous C3 activation due to an

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**FIG. 1.** Schematic diagram of the complement cascade reaction. Complement regulatory proteins are shown as shaded circles (fluid phase regulators) and boxes (membrane-associated regulators). MASP, MBL-associated serine protease; C1 INH, C1 inhibitor; SCPN, serum carboxypeptidase N; DAF, CD55; MCP, membrane cofactor protein (CD46); CR1, complement receptor 1 (CD35).
autoantibody, termed C3 nephritic factor (C3NeF), which stabilizes the labile C3bBb complex (8, 72). Here, identification of C3NeF is of significant importance.

HUS is characterized by microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure. There is compelling evidence that the atypical form of this fatal disease is often associated with a mutation in the C terminus of factor H, expressed as suboptimal regulator activity and consequently leading to hypocomplementemia (83). Occasionally, factor H is also reduced in cases of MPGN type II.

(iv) HAE. Hereditary angioedema (HAE) is an autosomal dominant condition with reduced concentration (type 1) or function (type 2) of C1 inhibitor (6, 9, 56). With respect to life-threatening consequences of edema formation, early diagnosis of the C1 inhibitor deficiency in these patients is extremely important. As spontaneous mutations can occur, a negative family history does not exclude the diagnosis. Furthermore, the penetrance differs significantly and minor symptoms or subclinical cases occur. The pathophysiology of HAE is complex, but it is now generally accepted that formation of bradykinin through activation of the kallikrein-kinin system, which is also controlled by C1 inhibitor, is the major inducer of the edema (58). The diagnosis is based on C1 inhibitor and C4 quantification. It is important to include both antigenic and functional assays for C1 inhibitor, since 15% of the patients have type 2 HAE with normal or even increased antigen concentration of C1 inhibitor. C4 is usually low in both cases, even between the attacks, and serves as a valuable supplement to C1 inhibitor analysis. The concentration of C3 is usually normal in HAE. All HAE patients described so far are homozygous in their deficiency, with less than 50% of the normal concentration and/or function. A homozygous C1 inhibitor deficiency is probably not compatible with life. Acute treatment requires purified C1 inhibitor or plasma transfusion, whereas androgens can be given prophylactically.

A clinical picture identical to HAE may occur in patients who develop autoantibodies to C1 inhibitor, in some cases related to hematologic malignancies (1). In addition to reduced C1 inhibitor and C4 levels, these patients frequently have a low C1q concentration in contrast to the HAE patients. Identification of this form of acquired angioedema is important, as its acute treatment requires higher doses of C1 inhibitor.

(v) Paroxysmal nocturnal hemoglobinuria. A somatic mutation in the gene coding for the phosphatidylinositol anchor results in a decreased expression of membrane proteins linked to this structure (5), including DAF (CD55) and CD59. Decreased expression of DAF and/or CD59 renders the red cells susceptible to complement-mediated lysis, which is the hallmark of this condition. Diagnosis is traditionally made by Ham’s test (acid lysis test) but is now specifically assessed by flow cytometric analysis of the respective cell surface proteins (31).

**COMPLEMENT TESTS**

(i) Functional assays. (a) Total complement activity of the classical, lectin, and alternative pathways. Hemolytic assays have traditionally been used to assess the functional activity of the complement system. They provide insight into the integrity of the entire cascade reaction. These tests are particularly useful in the investigation of suspected complement deficiencies.

Although described in numerous modifications, hemolytic assays are still based on protocols first described by Mayer (39) and Rapp and Borsos (63). Serial dilutions of the sample to be analyzed are incubated with antibody-sensitized sheep erythrocytes at a defined temperature. Hemolytic assays are performed either in tubes or in agarose plates. The results are usually expressed as reciprocal dilutions of the sample required to produce 50 or 100% lysis (CH50 or CH100, respectively). Tests evaluating the functional activity of the alternative pathway (AH50) use guinea pig, rabbit, or chicken erythrocytes as target cells (25). Here, activation of the classical pathway has to be blocked by adding EGTA to chelate Ca^{2+}, and an optimal concentration of Mg^{2+} is required. Detection of low or absent hemolytic activity in CH50 and/or AH50 directs further complement analysis.

However, AH50 is frequently normal or only slightly reduced in properdin deficiencies and needs to be replaced by alternative assays such as LPS activation assay or specific properdin tests (see below).

Simplified assays with a single serum dilution and a large number of erythrocytes are suitable for large-scale clinical screening, especially if they can be performed as semiautomated microassays (54). Commercially available cytolytic assays replacing erythrocytes with sensitized liposomes (36) offer certain advantages with regard to logistics, reproducibility, and suitability for automated systems.

The function of complement can also be tested by measuring the deposition of activation products upon activation of the serum with immobilized complement-activating substances (immunoglobulin M [classical pathway], LPS [alternative pathway], or mannose [lectin pathway]) on a microtiter plate. The combination of detection of C9 in the immunoglobulin M ELISA, properdin deposition in the LPS ELISA, and C4 binding in the lectin pathway assay excludes a suspected complement deficiency (12, 23, 59). It is likely that these tests will replace hemolytic assays in the future.

(b) Activity of individual components. Titration of individual complement proteins requires the addition of those components which are needed to complete the reaction sequence. A correct estimation of the components’ activity is obtained by calculation of functional molecules (63). The most convenient way to detect the functional activity of individual complement components is to test for the sample’s capability to reconstitute the hemolytic activity of a serum which is deficient for the protein. With the availability of complement-deficient or -depleted human sera, titration of individual complement proteins became much easier to perform. To finally prove that only one single component is lacking, a purified functionally active component can be added to the serum to restore hemolytic activity of the respective pathway.

Hemolytic assays are sensitive to in vitro activation of complement in serum. If serum is heat inactivated, has been stored for a long time at room temperature, or contains complement-activating agents (e.g., immune complexes or cold agglutinins), the hemolytic activity is reduced and may even be zero. Thus, fresh serum (<48 h old) should be used or samples should be stored at −70°C until tested.
(c) C1 inhibitor activity. Fifteen percent of patients with HAE have a dysfunctional protein (type 2 HAE) with normal or increased concentrations of C1 inhibitor antigen. To diagnose this form of HAE, it is mandatory to include a functional C1 inhibitor test (53, 81), which can be done by commercially available quantitative chromogenic assays.

(ii) Immunochemical assays for individual components. Individual complement components, irrespective of functional activity, can be measured by immunoprecipitation tests (radial immunodiffusion [RID] or nephelometry techniques), ELISA, or Western blotting. In routine diagnosis, C3 and C4 (and sometimes factor B) are most frequently measured, followed by C1 inhibitor, to verify the diagnosis of type 1 HAE. MBL and properdin quantification should now be considered included in the investigation of a suspected immunodeficiency. In most cases, where total hemolytic activities (CH50 and AH50) indicate a complement deficiency, immunochemical assays can be performed as an alternative to functional assays for the individual components. If a defect is verified immunochemically, further functional assays are not required. On the other hand, if immunochemical assays do not reveal any deficiency, the component may be functionally inactive and only a functional assay can verify the diagnosis.

(iii) Analysis of complement activation products. Total hemolytic activity and levels of individual complement components may also give some indication of an ongoing complement activation, e.g., low levels C4 and CH50 in active SLE. However, the same results may be obtained in cases with a genetic deficiency of components, e.g., C4, as frequently observed in SLE. Furthermore, in vivo complement activation in conjunction with an acute-phase reaction may leave the individual components within the normal range despite ongoing consumption, since most of the complement components are acute-phase proteins. In general, total hemolytic activity and individual component measurements are useful as first-level screening techniques, but they are not sensitive enough to detect pathologically increased complement activation in vivo. During the last two decades, highly specific monoclonal antibodies have been produced which recognize neoepitopes only exposed upon activation-induced conformational changes (42). This enables direct capture of the activation fragment by ELISA (44) or to high-capacity immunosorbents (16) without interference of the nonactivated component. These assays have today replaced the older generation of tests, which were hampered by preassay precipitation or fractionation steps or are time-consuming or of low capacity.

Complement activation products may be either split fragments after enzymatic cleavage of certain components, e.g., C4 (C4a and C4d), C3 (C3a and C3d), factor B (Bb), and C5 (C5a), or protein complexes, like C1rs-C1 inhibitor, the properdin-containing alternative pathway convertase C3bBbP, and SC5b-9 (soluble C5b-9 bound to S protein), where activated components are bound to their respective regulators.

Neoepitope-specific assays to detect fluid-phase activation have been described and are partly commercially available for the classical pathway (C1rs-C1 inhibitor, C4d, and C4bc), the alternative pathway (Ba, Bb, and C3bBbP), C3 (C3a, iC3b/ C3bc, and C3d) and the terminal reaction sequence (C5a and SC5b-9). Many of the neoepitope-specific antibodies can also be used to detect in situ complement activation by applying immunohistochemistry.

Complement activation products are usually present in only trace amounts in vivo, but they are rapidly generated in vitro (41). Therefore, it is crucial that samples are collected and stored properly to avoid in vitro activation. Blood should be drawn directly into EDTA-containing tubes at a final EDTA concentration of at least 10 mM. Alternatively, nafamostat mesilate can be used as an anticoagulant (60), whereas citrate and heparin do not sufficiently block complement activation. The sample should be cooled, and plasma should be prepared immediately and stored at −70°C.

The various complement activation products have different half-lives in vivo. This is important for the activation product(s) of choice to be measured. Due to rapid receptor binding, the biologically highly active and important C5a fragment has a half-life of approximately 1 min (57) and is difficult to detect in samples obtained in vivo, whereas the various C3 activation products are readily detectable due to half-lives of a few hours (74). The half-life of SC5b-9 is 50 to 60 min (45). SC5b-9, in contrast to C3 activation products, is relatively stable in vitro and is a reliable indicator of terminal pathway activation.

The amount of an activation product should be related to the concentration of the native component, since a low level of native component would yield smaller amounts of activation products during in vivo activation. Thus, it has been postulated that the ratio between the activation product and the native component is a more sensitive indicator of in vivo activation than the activation product alone (55).

(v) Assays for complement-binding autoantibodies. C3NeF can be measured in a decay experiment by stabilization of the alternative pathway convertase C3bBb (8, 24). A semiquantitative screening assay based on a stable cell (sheep erythrocyte)-bound convertase was reported by Rother (67). Fluid-phase conversion of C3 upon the mixture of normal serum and C3NeF-containing patients’ serum can be visualized in an immunofixation assay (also commercially available).

Autoantibodies to C1q or to C1 inhibitor can be detected by ELISA with purified proteins immobilized on the microtiter plate. Interpretation of results from various dilutions of patients’ serum or plasma should be done in comparison with data received from a large panel of healthy controls (71). In a C1q autoantibody ELISA, sample dilution requires the presence of a high salt concentration to avoid immune complex binding.

**COMPLEMENT ANALYSIS IN EXPERIMENTAL SETTINGS**

(i) In vitro experiments with human serum and blood. Activation mechanisms and intervention of complement activation can readily be studied in vitro with human serum. Since there are no cells present and there is no biological turnover, any activation product including C5a can be detected. A more physiological approach, however, is to use whole human blood. In this case, anticoagulants need to be added. However, most of the anticoagulants, such as EDTA, citrate, and heparin interfere with complement activation and should be avoided. From our own experience, recombinant hirudin (lepirudin), a highly specific thrombin inhibitor, does not affect complement
and seems particularly useful for complement studies with whole human blood (40).

To avoid further activation during preparation and storage after the experiments have been performed, it is necessary to add EDTA (optimal in combination with nafamostat mesilate or futhan) at the end of the experiments (60).

In vivo, experimental studies with human complement are limited for obvious reasons. However, certain clinical conditions can be mimicked by experimental settings, e.g., extracorporeal circulation (hemodialysis or cardiopulmonary bypass), and have proved useful for the study of complement activation and possible effects of complement intervention.

(ii) Animal experiments. (a) Application of gene-deficient or knockout animals. A number of animals with genetic deficiencies of individual complement components have been described (66) and are commercially available (e.g., C2-, C4-, or C3-deficient guinea pigs, C3-deficient dogs, C5-deficient mice, and C6-deficient rats and rabbits). During the last few years, an additional number of knockout mice have been generated (e.g., deficient for C1q, C3, C4, and factor B) (2). These animals are particularly useful for the study of the importance of complement to various diseases and for delineating activation mechanisms and the pathways of activation involved. In principal, reconstitution experiments with purified components should be included, but for practical reasons, this is not always possible, since limited numbers of components have been purified from the different species. The addition of a component from another species may be valuable, with a functional cross-reactivity provided. Administration of a purified human component to a gene-deficient mouse may work with respect to some complement effects, such as lysis, but molecular incompatibility may imply that different receptor-mediated effects cannot be studied. Reconstitution with whole serum of the same species may serve as an alternative.

(b) Complement depletion. Cobra venom factor (CVF) has been used for decades to deplete complement animals. CVF is analogous to C3b and activates C3 but is resistant to regulation by human factors I and H (75). CVF treatment is frequently and misleadingly called inhibition of complement; in fact, an extreme activation with subsequent complement consumption takes place. The animals usually tolerate this activation (fairly) well, since only a limited activation of the terminal pathway occurs. Nevertheless, adverse effects from CVF have been reported (38, 64), and one should be very cautious with the interpretation of the data from such experiments. A direct inhibition of complement at a certain level is recommended instead of CVF treatment when mechanisms of complement activation are to be studied.

(c) Testing animal complement. Hemolytic assays can be designed for any species. However, information obtained from such assays is limited but still valuable. They can be used to monitor the effect of complement inhibition by a certain substance or as a semiquantitative measure of complement activation under controlled conditions, since hemolysis will be lowered proportionally to the consumption of complement. In many animal species, a comprehensive analysis of complement is still hampered by the lack of suitable reagents and assays. A major factor affecting the evaluation of complement activity in the serum of previously unstudied species is the choice of an appropriate target cell. Low or nondetectable classical pathway activity of murine or bovine complement, for example, results from applying conventional methods with sensitized sheep erythrocytes. Comparison of classical pathway activity between different species is difficult to evaluate, since hemolytic titers are also influenced by the source and quality of erythrocyte-specific antibodies (19). Applying optimal test conditions, it can be demonstrated that virtually all species of laboratory and domestic animals possess classical as well as alternative pathway activity (3, 21, 73). The compatibility of components within the cascade sequence between different species and guinea pigs or humans often allows the titration of individual components (3, 80). To evaluate the pathophysiological contribution of complement to certain diseases, assessment of complement activation by immunochemical means is indispensable. However, as measurement of components is not a sensitive and reliable indicator of a slight activation, activation products should be tested. Whereas a number of neoepitope specific assays have been described for human complement activation, only a few are available for animal studies. These are either assays for human activation products which cross-react with other species or assays particularly designed for animal studies. The latter include assays based on monoclonal antibodies to neoepitopes of guinea pig C5a (32) and C3 activation products (17), rabbit C5a (4), and rat C5b-9 (69). Several of the human neoepitope-specific assays cross-react sufficiently well with other species, like baboon C3bc (20); baboon C3bc, C5a, and C5b-9 (43); baboon C4d, C5a, Bb, and C5b-9 (13); and porcine C5b-9 (22).

The possible species cross-reactivity of a certain assay made for human complement activation can easily be checked by activating the serum from the respective species by, e.g., zymosan, and comparing it to the nonactivated control. If the signal in activated serum is stronger than in the nonactivated serum, the assay cross-reacts with this species and the degree of cross-reactivity can be estimated.

REFERENCES


