

The Complement System: Its Importance in the Host Response to Viral Infection

ROBERT L. HIRSCH

Howard Hughes Medical Institute, Department of Neurology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

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INTRODUCTION

The complement system is composed of a series of serum proteins that function in a variety of specific and nonspecific immune defense mechanisms. Upon interaction with activators, the complement system initiates or amplifies events that might normally occur at low levels. Activation of the complement system can be viewed as a series of enzymatic effects which result in limited proteolytic events, generation of fragments, and biologically significant occurrences, which in some manner modify the activating substance or its environment. The activating substance or particle, as the case may be, is either directly affected by the activation or is prepared for other arms of the immune response.

It is not the purpose of this review to detail the specific proteins and regulatory mechanisms involved in the activation of the complement system, but rather to review what is known about the interaction of the complement system with viruses and the role of the complement system in host defense against viral infection. Nonetheless, a brief summary of the salient features of the complement system is a requisite for a critical discussion of the interaction of the complement system with viruses.

THE COMPLEMENT SYSTEM: COMPONENTS AND ACTIVATION

The complement system can be activated via two mechanisms, the classical and alternative

pathways. Although these pathways share many common features and the biological result of activation of each pathway can be the same, the pathways are quite different.

Activation of the Classical Pathway

The activation of the classical pathway is most often initiated by the binding of the first component of complement (C1) to antigen-antibody complexes; however, other molecules, including polyanions, viral proteins, and lipid A of lipopolysaccharides, may also mediate classical pathway activation in the absence of antibody. C1 exists in the serum as a calcium-dependent complex of three subunits C1q, C1r, and C1s. In situations involving antigen-antibody complexes, C1q binds to the Fc region of immunoglobulin in the complexes. This binding initiates the classical complement pathway cascade (Fig. 1). After the union of C1q to the antigen-antibody complex, C1r activates C1s to become activated C1s (C1s[~]; by convention, a superscript bar designates an active enzyme of the classical pathway) (reviewed in reference 20). This protease (C1s[~]) cleaves C4 to yield a small-molecular-weight fragment, C4a, and the major cleavage fragment, C4b. The presence of C4b allows C1s to cleave C2, the major cleavage product of which is C2a. C2a binds to C4b in the presence of magnesium, and the C4b2a complex thus formed is known as the classical pathway C3 convertase. The C3 convertase allows for subsequent cleavage of C3 into C3a and C3b, and the

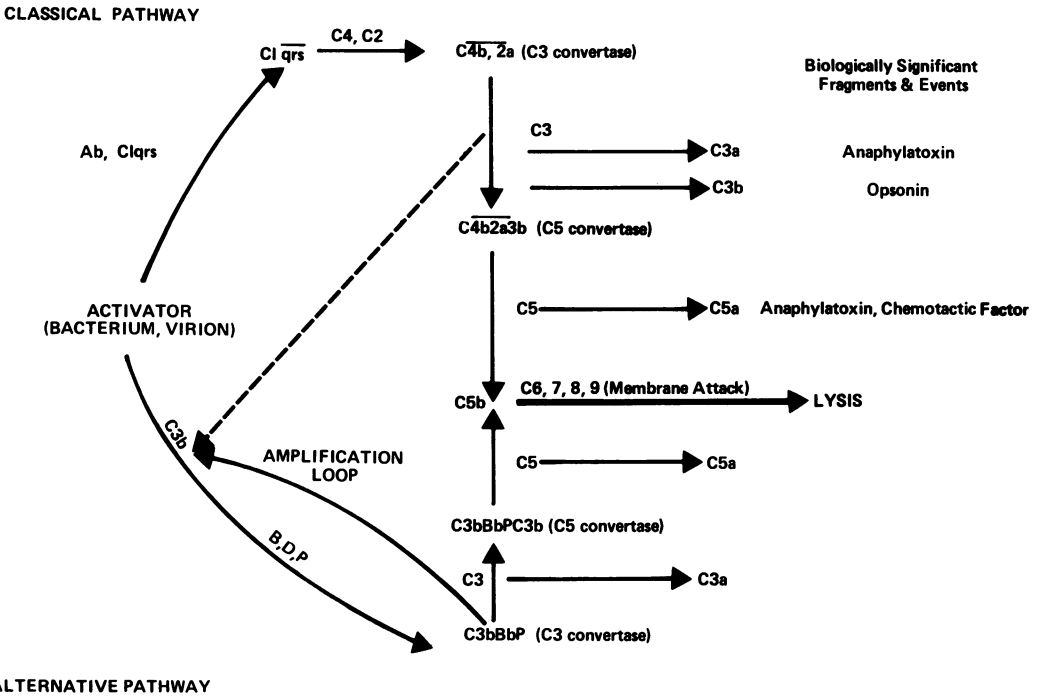


FIG. 1. Schematic representation of the complement system showing the two pathways by which microorganisms initiate complement activation and generate biologically important events. Binding of immune complexes to C1q via the Fc portion of an immunoglobulin molecule activates the classical pathway. Activated C1 (C1qrs) then activates C4 and C2. The bimolecular complex (C4b2a) in turn cleaves C3 into two fragments, C3a and C3b. C3b can either form part of the enzyme which cleaves C5 and initiates the membrane attack complex or it can bind to microbial surfaces and act as an opsonin. When tested *in vivo*, with the use of purified complement components, activation of the ACP does not require immunoglobulin. The binding of C3b (generated either by the inefficient interaction of C3, B, and D in the fluid phase or by action of the classical pathway C3 convertase) to a microbial surface initiates the ACP. The fixed C3b binds factor B and, unless stabilized by properdin (P), undergoes intrinsic decay. When stabilized, however, the C3bBb complex is an efficient enzyme and cleaves further C3 molecules (amplification loop). When an additional C3b binds to the C3bBbP complex, it acquires C5 cleaving capability and generates the membrane attack complex.

activation of the terminal lytic mechanism of the complement system (see below). The classical pathway C3 convertase is under at least three forms of control. First, the enzyme is unstable at 37°C; second, C3b inactivator (I) in the presence of a cofactor, C4b binding protein, degrades C4b in the fluid phase, causing accelerated decay of the convertase; and finally, C4b binding protein in the presence of I removes C4b from the surface to which it is bound (17).

Activation of the Alternative Pathway

The alternative or properdin pathway of the complement system is essentially a bypass system for the classical pathway, and its activation probably does not require (but may be enhanced by) the presence of immunoglobulins. For this reason and because of the fact that bacterial cell surfaces and viruses are among the activators of the alternative complement pathway (ACP), it is

likely that the ACP evolved as a defense mechanism prior to the classical pathway. An essential feature of the ACP is the ACP amplification C3 convertase. Upon binding of C3b, which is generated by either the classical pathway or low-grade fluid-phase ACP activation, to an activating surface, factor B binds to C3b to form C3bB complexes (Fig. 1). Factor D cleaves factor B to form C3bBb, which is the ACP C3 convertase. This process occurs at low levels in the fluid phase, but is amplified and controlled when C3b binds to the surface of a particle. Properdin (P), like C3 nephritic factor (C3NeF, an anti-C3bBb globulin present in the serum of patients with glomerulonephritis), stabilizes the complex such that further cleavage of C3 (amplification) can occur via the ACP C3 convertase (C3bBbP or C3bBbC3NeF). The C3bBb enzyme is controlled by four mechanisms. The complex is unstable, and therefore intrinsic decay occurs

readily at 37°C. H, a serum glycoprotein, can bind to C3b and impair the binding of B; H can increase the rate of Bb dissociation from C3b; and H can increase the susceptibility of C3b to the action of I. The action of H and I on the C3bB complex depends on its ability to bind to C3b, which is directly related to the levels of sialic acid on the particle surface. It is likely that surfaces with low levels of sialic acid are able to protect, in some manner, the ACP C3 convertase from degradation by H and I. Thus, the ability of a particle to sustain ACP activation is inversely related to the amount of surface sialic acid (16). When H does bind to the C3bB complex, C3b is exposed and the cleavage of the convertase by I is enhanced, resulting in dissociation of the complex to C3bi (cleaved and inactive form of C3b) and Bbi (inactive Bb, unable to hydrolyze C3 and C5) (16).

Terminal Lytic Events

As outlined above, C3 can be cleaved by either the classical C3 convertase (C4b2a) or by the ACP C3 convertase (C3bBb). The C4b2a3b enzyme is formed when C3 is cleaved via the classical pathway. This enzyme cleaves C5 into C5a and C5b. C6 binds to and stabilizes C5b (otherwise, dissociation of C5b from the C4b2a3b enzyme occurs). The C5b6 complex then reacts with C7 to form a trimolecular complex, C5b67. Upon interaction of this complex with C8 and C9 (for example, on the surface of an erythrocyte), lysis occurs. The mechanism of the lytic attack through the classical pathway has been reviewed elsewhere (34, 35). The initiation of the lytic mechanism through the ACP occurs when the ACP C3 convertase, upon stabilization by properdin and binding of an additional C3b, acquires the ability to cleave C5. The remaining steps in the lytic mechanism are presumably the same as those in the classical pathway (55).

An important event of biological significance associated with activation of the complement system is the generation of the membrane attack complex and subsequent lysis of lipid bilayers (membranes). However, the generation of several complement fragments as a result of complement activation is also of great biological importance (Fig. 1). The C3a and C5a fragments can induce histamine release, and C5a can also promote leukocyte chemotaxis. C3b and, to a lesser extent, C4b are opsonins and, when bound to the surface of a microbe, can facilitate the binding of microbes to lymphocytes or phagocytic cells. The Bb fragment of factor B of the alternative pathway can induce macrophage spreading and inhibit macrophage mobility, whereas the Ba fragment can induce chemotaxis of polymorphonuclear leukocytes. Other recent reviews have detailed the biological activi-

ties of complement components and fragment (27, 53).

INTERACTION OF COMPLEMENT WITH VIRUS AND VIRUS-INFECTED CELLS IN VITRO

Over the past 50 years, scattered reports in the literature have indicated that the complement system, either independently or in the presence of antibody, plays an important role in host defense against virus infection. Douglas and Smith showed that a heat-labile component of rabbit serum enhanced the interaction of vaccinia virus with the cellular elements of blood (14). Mueller demonstrated that fresh guinea pig serum enhanced the activity of immune serum against Rous sarcoma virus and suggested that this enhanced activity was due to complement (40). Morgan (39) and Whitman (67) showed that unheated rabbit serum, containing antibody to western equine encephalomyelitis virus, had a higher neutralizing antibody titer than serum heated at 56°C for 30 min. Furthermore, addition of unheated fresh serum from several species increased neutralizing antibody titers. These were the first of many studies indicating that a heat-labile serum factor was required for or enhanced antibody-mediated virus neutralization.

Antibody-Dependent, Complement-Enhancing Neutralization of Viruses

The neutralization of virus by antibody is a multifaceted reaction which is dependent upon the virus, the class and concentration of immunoglobulin, and the host cell under study (11). Antibody may prevent viral adsorption to a cell surface by covering critical sites, by causing aggregation, or by altering the charge of a virus. Antibody may also influence infection of a cell after virus adsorption to a cell surface by inhibiting virus penetration or uncoating of viral nucleic acid, or both (11). Antibody may also enhance uptake and phagocytic destruction of virus by a cell. Since antibody may influence viral infectivity by several mechanisms, the complement system may also act to enhance neutralization in several ways.

Virolysis. One mechanism by which complement has been shown to enhance antibody-mediated virus neutralization is by virolysis, actual destruction of virion integrity. This phenomenon has been demonstrated for several different classes of enveloped viruses. Electron microscopic evidence for virolysis was first presented by Berry and Almeida, using avian infectious bronchitis virus and a rabbit anti-avian infectious bronchitis virus serum (5). Stollar demonstrated that an antibody-coated togavirus,

Sindbis virus, when exposed to a complement source, was lysed, as determined by the release of the radioactively labeled viral ribonucleic acid (RNA) (60). Biochemical and electron microscopic studies showed that antibody and complement also lysed an arenavirus, lymphocytic choriomeningitis virus (LCMV) (66). Studies on the neutralization of equine arteritis virus (EAV) by late (immunoglobulin G, IgG) antibody raised in several species demonstrated that neutralization was dependent upon complement in that only partial neutralization occurred after a prolonged incubation in the absence of complement (50). The release of labeled RNA from antibody-coated EAV occurred upon addition of equine or guinea pig complement (52), indicating that the enhanced neutralization by late antiserum proceeded through virolysis. However, other evidence indicated that complement interacted with IgG-sensitized EAV to induce structural alterations in the absence of virolysis. Complement-antibody-neutralized EAV complexes were treated with trypsin, and 32% of original EAV infectivity was recovered. Subsequently, 89% of the recovered infectivity was destroyed by treatment with ribonuclease, suggesting that a fraction of the EAV suffered structural damage yet was still infectious after treatment with complement. Control, non-complement-treated, sensitized EAV, when treated with anti-IgG and subsequently treated with trypsin, were resistant to ribonuclease and fully reneutralizable with anti-IgG serum (51). Thus, it appears as if complement, through the action of the terminal membrane attack complex, can lyse antibody-coated enveloped virions as well as cause major alterations in the virion envelope in the absence of complete virolysis.

Complement-enhancing neutralization without virolysis. The requirement for complement in antibody-mediated neutralization depends upon the class of immunoglobulin used to sensitize virus. For example, in studies with herpesviruses, early antiserum, containing IgM antiviral immunoglobulin, requires complement for any significant neutralization of herpes simplex virus (HSV), whereas late antiserum, containing IgG antibody, does not require complement (59, 70); complement may, however, enhance IgG-mediated neutralization (69).

In the studies of avian infectious bronchitis virus cited above (5), rabbit antiserum caused virolysis. However, unheated homologous (fowl) antiserum was shown to induce greater neutralization than heated serum in the absence of typical complement lesions on the surface of virions. The enhanced neutralization of avian infectious bronchitis virus in the presence of complement correlated with electron microscopic findings: virions treated with unheated serum

demonstrated a protein halo of larger diameter than did virions treated with heated serum. Thus, these studies indicate that complement can enhance neutralization by contributing extra protein on the virion surface.

Studies in other systems have been performed to evaluate other mechanisms by which complement could enhance neutralization of virus in the absence of virolysis. Serum deficient in either early or late complement components was used to evaluate the requirements for complement in viral neutralization. It was shown that C4-deficient guinea pig serum did not neutralize IgM-HSV complexes, whereas C5- or C6-deficient sera did neutralize the IgM-HSV complexes (12). (Table 1 indicates several types of sera or serum treatments used to evaluate the contributions of the classical and alternative pathways in biological phenomena.) These studies indicated that completion of the terminal lytic phase of the complement system was not always required for complement-induced neutralization of antibody-virus complexes. Furthermore, when functionally purified complement components were used, sequential addition of C1, C4, C2, and C3 to IgM-HSV complexes caused sequential decreases in infectivity. Thus, studies with the IgM-HSV infectious complexes showed that virolysis was not a prerequisite for neutralization induced by complement (12). Similar studies with Newcastle disease virus also showed that virolysis was not required for complement-mediated neutralization (33).

More recent studies have also demonstrated that the late components of complement (C5 or C6) are not required for enhancement of IgG-mediated neutralization of virus (31). Studies using sera deficient in the early components of complement (C1r, C4, C2, or C3) showed that C1 through C3 were responsible for enhancement of IgG-mediated neutralization of both vaccinia virus and vesicular stomatitis virus (VSV) (31). Detailed studies have evaluated the interaction of VSV with the complement system. Activation of the classical pathway by VSV resulted in significant viral neutralization in 50% fresh, but not heat-inactivated, human serum (37, 38, 61). Antibody in human serum was initially thought not to be required for neutralization in that immunoglobulin isolated from human serum failed to neutralize VSV. In addition, serum absorbed with VSV-infected or uninfected cells, to remove antibody, still possessed neutralizing activity, and C3b was deposited on the surface of the virus when serum from a patient with agammaglobulinemia was tested. However, it was noted that the serum from the patient with agammaglobulinemia did not neutralize the virus, and this suggested that immunoglobulin may have been im-

TABLE 1. Common methods for evaluating contributions of complement to biological phenomena

Material	Effect
Serum treatment or source	
Heat (56°C, 30 min)	Destroys hemolytic complement activity
EGTA ^a -Mg ²⁺	Selective chelation of Ca ²⁺ inhibits the classical pathway
C4D-GPS	Serum from guinea pigs with an inherited deficiency of C4; isolates the ACP
C2D-HS	Serum from patients with inherited deficiency of C2; same use as C4D-GPS
C6D-HS, C7D-HS, C8D-HS	Serum from patients with inherited deficiencies of C6, C7, or C8; used to eliminate the membrane attack complex
Animal sources and treatment	
B10.D2 old line	Mice with an inherited deficiency of C5 and identical to B10.D2 new-line mice except for this trait
C4-deficient guinea pigs	Guinea pigs with an inherited deficiency of C4
COVF	A protein from cobra venom which temporarily depletes animals of C3 through C9 by virtue of the fact that COVF is analogous to mammalian C3b but is insensitive to C3b inactivator

^a Ethylene glycol-bis(β -aminoethyl ether)-*N,N*-tetraacetic acid.

portant for neutralization. The ACP was not required but may have enhanced neutralization (factor B-depleted serum possessed lower neutralizing activity), and the terminal complement components (C5 through C8) were not required for neutralization (C5-deficient serum and C6-, C7-, or C8-depleted serum retained significant neutralizing capacity). C1q-deficient, C4-depleted, or C2-deficient serum did not support neutralization of VSV, and upon addition of the missing component to these sera, neutralizing activity was restored. In addition, direct evidence for activation of the classical pathway was presented (38), suggesting that neutralization occurred via antibody-independent activation of the classical pathway. However, it was also demonstrated that addition of purified C1, C2, C3, C4, and C5 did not neutralize VSV, further suggesting that an additional serum factor was required for neutralization (38). Recent studies have in fact shown that anti-VSV IgM present in all sera tested was the additional factor required for neutralization of VSV (4).

A number of mechanisms may be envisioned as contributing to the complement enhancement of antibody neutralization of enveloped viruses. As noted above, the interaction of antibody-coated virions with the full complement cascade may result in virolysis. As shown for EAV, structural damage may also occur in which critical sites of the virus are destroyed. Complement components may also increase the amount of protein on the surface of the virion, thus interfering with the binding of viruses to target cells or altering the surface charge of the virus. Finally, in situations in which neutralization occurs after virus penetration of a target cell, complement may enhance the uptake and destruction of virus within the cell.

The effect of complement on the neutralization of nonenveloped viruses has also been

studied. Early studies on poliovirus (WS strain) showed that neutralizing antibody titers were not influenced by the presence of complement, in that heating of serum at 56°C for 30 min failed to decrease antibody titers (32). More recent studies using polyoma virus have shown that the early complement components (C1 through C3) and not C5 through C7 enhanced neutralization of virus-antibody complexes (41). Furthermore, cleavage of C3 to C3b seemed to be necessary for enhanced neutralization, as the presence of C2 was required. Since the sedimentation rate of labeled virus was shown to increase upon addition of complement, the enhancement of polyoma virus neutralization by complement seemed to proceed through agglutination of the virus-antibody-complement complexes.

Antibody-Independent Activation of Complement by Virus

The demonstration by Douglas and Smith (14) of a heat-labile, calcium-dependent, virus-inhibitory serum factor went unnoticed until the late 1940s, when Ginsberg and Horsfall reported that serum obtained from humans, guinea pigs, or rabbits could neutralize mumps virus, Newcastle disease virus (NDV), and influenza A and B viruses (18). The factors responsible for neutralization were inactivated upon heating (56°C for 30 min) and upon prolonged storage at 4°C. The virus-serum component combination was calcium dependent, did not undergo spontaneous dissociation, and was only partially reversible by heating mixture for 24 h or by removal of calcium. The authors did not think that this serum component was related to hemolytic complement. At the same time that this report appeared, Howitt made a similar observation that NDV was neutralized by normal serum (26). The characteristics of this serum factor were similar

to those reported by Ginsberg and Horsfall (18), and this factor was speculated by Howitt to be associated with serum complement activity. In retrospect, the neutralization may have been due to the presence of natural antibody (64), and the neutralizing activity may have been dependent on complement (e.g., antibody-mediated, complement-requiring neutralization).

Regardless of the nature of the factor responsible for the observed neutralization of virus by normal sera, the results inspired Pillemer and his co-workers to investigate the influence of the properdin system (ACP) on virus infectivity (63). At this time, Pillemer and his colleagues had observed that the properdin system was capable of causing the lysis of certain erythrocytes, bacteria, and protozoa. It was shown that 50% fresh human serum inhibited both infectivity and hemagglutination of NDV. The activity was heat labile, and properdin was required (e.g., zymosan-treated serum was ineffective in inhibiting infectivity or hemagglutination). The maximum inhibition of infectivity (100-fold reduction) produced by properdin occurred at the concentration range for properdin observed in fresh serum. Although at this time in the history of the complement system there were only four recognized components, sera depleted of any of these components eliminated the inhibitory effect. Magnesium was also shown to be required for inhibition of virus infectivity. In contrast to earlier work in which calcium was shown to be required for virus inhibition (18), these studies showed that only magnesium was required for inhibition of hemagglutination activity. In addition, calcium did not enhance the virus inhibition observed in the presence of magnesium. Finally, in comparison to properdin levels in fresh serum incubated with normal allantoic fluid, the levels of properdin were significantly reduced in serum incubated with allantoic fluid containing NDV. Thus, these first detailed studies showed that complement could interact with virus in the absence of detectable antiviral antibody and cause virus inactivation. Although it is likely that much of the antiviral activity was complement mediated and independent of antiviral antibody, it is possible that natural antibody to host membrane components of chick cells, in the presence of complement, may have contributed to and enhanced the reported actions of the properdin pathway on NDV. It should also be noted that a similar report on the interaction of fresh human serum with T2 bacteriophage suggested that the properdin system was involved in neutralization of this bacterial pathogen (62).

Several recent studies have now confirmed and extended the work reported by Wedgewood and colleagues (63). After incubation of either

LCMV or NDV in 50% fresh human serum, it was shown that, in the absence of detectable antibody, significant reduction in virus infectivity occurred (64). Several sera and serum treatments were employed to determine the mechanism by which NDV and LCMV were inactivated. In the case of NDV, significant inactivation of virus could be demonstrated in C4-depleted human serum, indicating that NDV was inactivated by the action of the ACP. However, factor B-depleted human serum could also cause significant reduction of infectivity, indicating that the classical pathway, in the absence of antiviral antibody, could also be utilized. Furthermore, sera from four patients with agammaglobulinemia were also effective in reducing NDV infectivity. However, the ability of fresh human serum to inactivate NDV was dependent upon the cells in which the virus was grown. If grown in chicken embryo fibroblasts, the above characteristics for serum inactivation of NDV held true. If, however, the virus was grown in a human cell line, HeLa cells, fresh serum was incapable of causing virus inactivation. This result suggested that antibody to host cell components may have contributed to the observed inactivation of NDV grown in chick cells; however, sera absorbed with chick cells continued to inactivate virus (64). Thus, these studies and those concerning VSV mentioned above (61) suggest that the passage history of virus may determine its ability to be inactivated by complement, and this factor may play a role in determining viral virulence. The case of serum inactivation of LCMV was quite different (64). In these studies host modifications of the virus also played a significant role in determining the extent of inactivation; however, it was shown that the presence of natural antibody to the host cell components determined the degree of viral inactivation. That is, LCMV assimilated host cell antigens during the production of virus, and antibodies directed against these antigens determined the degree of virus inactivation (64).

In addition to studies of NDV, in which indirect evidence (viral inactivation) for complement activation was presented, studies with other viruses have directly shown that the classical pathway can be activated independently of detectable antiviral or anti-host cell antibody. It was initially observed that a number of different RNA tumor viruses could be lysed by fresh human serum (65), but not by serum from other species (guinea pig, feline, murine, or simian). The assay used employed the release of the RNA-dependent deoxyribonucleic acid polymerase from virions after incubation of virus in 50% human serum. Normal fresh human serum, but not C2-deficient, C4-deficient, C2-depleted, or C4-depleted human serum or heated serum

(56°C for 30 min), caused release of (RNA-dependent deoxyribonucleic acid polymerase), or significant reductions in infectivity or both (65). Although no antibody to virus was detected in the serum tested, a requirement for heat-labile antibody-dependent virolysis could not be completely ruled out; however, serum absorbed with cells expressing retrovirus antigen and serum from patients with agammaglobulemia were also effective in causing virolysis. Finally, heated human serum was unable to sensitize virions for lysis by fresh guinea pig complement. Since only human serum and not serum from nonprimates lysed oncornaviruses, these studies suggested that complement may play a role in limiting transmission of type C retroviruses in humans (65). Studies by others, however, using similar methods, i.e., release of RNA-dependent deoxyribonucleic acid polymerase from murine leukemia virus (MuLV) and primate retroviruses, showed that fresh sera from cats and rabbits, as well as humans and Old and New World monkeys, lysed type C retroviruses (56).

Further studies were performed to detail the interaction of human complement with retroviruses (3, 10). These studies showed that C1q bound directly to the envelope of oncornaviruses in the absence of immunoglobulins (10) and that complement components were deposited on the virion surface. In addition to the observed activation of the classical pathway by retroviruses, reduced lysis of MuLV was observed in factor B-depleted serum, suggesting that the ACP amplification C3 convertase loop may have been required for effective virolysis. Subsequent studies show that the ACP could be activated by MuLV, but activation of the ACP required approximately 5,000 times more virions than for activation of the classical pathway (3). The nature of the C1q receptor for MuLV was determined to be the p15E virion protein (3). Subsequent studies showed that the inability of serum from guinea pigs to lyse MuLV was not due to a defect in the binding of guinea pig C1q to virus but rather to the inability of guinea pig C1s to be activated (2).

In addition to NDV and MuLV, Sindbis virus has recently been shown to activate both the classical complement pathway and the ACP in the absence of detectable antibody. In these investigations it was observed that gradient-purified Sindbis virus (as little as 10 µg/ml), propagated in either hamster or chicken fibroblasts, consumed C3 when incubated in normal human serum or ethylene glycol-bis(β-aminoethyl ether)-*N,N*-tetraacetic acid-magnesium-treated human serum, as well as in normal guinea pig serum and C4-deficient guinea pig serum (Fig. 2) (25). These results indicated that Sindbis virus activated, at least, the ACP. Fur-

ther studies showed that C4 was also consumed in normal human serum, indicating that the classical pathway was also activated by Sindbis virus. The observed activation required little or no antibody in that C3 and C4 were consumed in serum from three patients with agammaglobulinemia, in serum absorbed with infected or uninfected host cells in which the virus was produced, and in serum containing no detectable neutralizing antibody to Sindbis virus. In contrast to the studies with MuLV, guinea pig serum supported C3 consumption by both the classical pathway and the ACP, and unlike the studies of MuLV and VSV, activation of both pathways was evoked at similar concentrations of virus.

In studies of C3 consumption by Sindbis virus, it was shown that, as with the other viruses discussed above (NDV and VSV), the host cell of origin influenced the ability of Sindbis virus to activate complement. No differences in classical pathway activation by Sindbis virus grown in chick or hamster cells were observed; when the ACP was evaluated, virus grown in chick cells did consume more C3 than did virus grown in hamster cells (Fig. 2) (25). Recent studies have demonstrated that the differential activation of the ACP by Sindbis virus propagated in different host cells can be related to the host-determined sialic acid content of the virus (24a; R. L. Hirsch, D. E. Griffin, and J. A. Winkelstein, *Fed. Proc.* 40:966, 1981). Virus grown in a mosquito cell line and lacking detectable sialic acid activated the ACP much better than did virus grown in baby hamster kidney cells and containing large amounts of sialic acid. Furthermore, the hamster virus, when treated with neuraminidase to remove sialic acid, became a much more effective activator of the ACP (Table 2). These results are in agreement with those of Fearon (16), who has shown that high levels of sialic acid on a particle favor the interaction of H with the surface-bound C3bBb enzyme. This interaction results in dissociation and inactivation by I of the C3bBb complex, effectively inhibiting the ACP. Thus, the antibody-independent activation of the ACP by Sindbis virus is influenced by host origin of the virus. Since viral sialic acid is host determined, these studies suggest that the activity of the complement system and its effects on viral pathogenesis depend, in part, upon the genetic constitution of the infected host and the host's ability to modify the infecting virus.

Antibody-Dependent and -Independent Interaction of Complement with Virus-Infected Cells

In addition to the ability of the complement system to interact with free virus in the presence

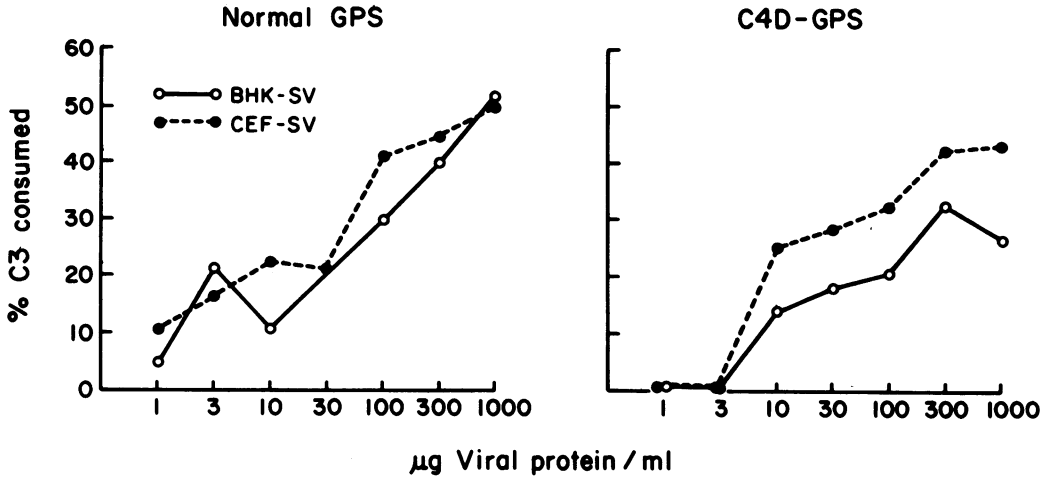


FIG. 2. Activation of complement by purified Sindbis virus (SV). SV produced in hamster (BHK) and chick (CEF) cells consumes similar amounts of C3 in normal guinea pig serum (GPS); however, in C4-deficient GPS(C4D-GPS), when the ACP is isolated, virus made in CEF consumes significantly more C3 ($P < 0.05$, at 1 mg/ml). This difference may be due to differences in the host-determined sialic acid levels of the virions produced by these cells. (Reprinted from reference 25 with permission from the Williams & Wilkins Co., Baltimore, Md.)

or absence of antibody, the complement system can also interact with viral antigens expressed on the surface of cells. The interaction of complement and antibody with infected cells may result in cell lysis. The conditions necessary for complement-mediated lysis of infected cells have recently been reviewed in detail (45, 46). However, important features will be summarized here.

Lysis of virus-infected cells by complement and antibody requires that sufficient viral antigen be expressed on the surface of the cell and

that the antigen be present in a form and configuration recognizable by antibody (6). An extremely large number of antibody molecules need to be bound to the surface of an infected cell prior to lysis. This could be a result of one or more factors. For example, the subclass of immunoglobulin which binds to the viral antigens may be inefficient in activating complement, or the antigens expressed on the surface of the cell may not bind to complement-fixing antibody with high efficiency. The requirement for sufficient expression of viral antigen has been demonstrated with cells infected with different strains of measles virus (30). Acutely infected cells bound two to three times more antibody and were more susceptible to complement-mediated, antibody-dependent lysis than were persistently infected cells.

The specificity of complement-mediated, antibody-dependent lysis of infected cells has also been addressed. It was demonstrated that, after an individual was vaccinated with mumps virus, antibody developed which, in the presence of complement, could lyse mumps virus-infected cells (49). Evidence for the specificity of this phenomenon has also been presented for measles virus vaccination (45).

Further studies with the measles model for complement-mediated, antibody-dependent lysis of infected cells have shown that lysis occurs through $F(Ab')_2$ -mediated activation of the ACP. Participation of the classical pathway was not necessary for lysis (29) Studies using various treatments of serum to eliminate classical pathway activity have shown that there was no reduction in the efficiency of complement-medi-

TABLE 2. Effect of virion sialic acid content on activation of the ACP^a

Source of Sindbis virus	Sialic acid content (nmol/mg of protein)	Viral protein (μ g/ml)	% C3 consumption by virus in C4D-GPS (\pm SD)
Baby hamster kidney cells	10.2	800	49 \pm 6
		280	40 \pm 9
		80	34 \pm 1
Baby hamster kidney cells (virus treated with neuraminidase)	2.0	800	76 \pm 12
		280	67 \pm 10
		80	59 \pm 12
Mosquito cells	<2.0	800	75 \pm 4
		280	69 \pm 4
		80	57 \pm 3

^a Purified Sindbis virus, from the indicated host source, was incubated in 10% C4-deficient guinea pig serum (C4D-GPS) (to isolate the alternative pathway), and after 30 min at 37°C, the amount of C3 consumed was ascertained by a standard hemolytic assay. (Data from three individual experiments; 24a.)

ated, antibody-dependent lysis of infected cells. However, removal of factor B or properdin completely inhibited lysis of measles-infected cells, and upon addition of these components back to the serum, lytic activity was recovered (46). The requirement of magnesium, but not calcium, for effective lysis also implied that the ACP was responsible for the lysis of infected cells (29). More recently, lysis of antibody-sensitized, measles-infected cells has been accomplished by adding to the cells all of the purified components of the ACP (58). Under these conditions, it was demonstrated that activation of the ACP was initiated by the virus-infected cells, in the absence of antibody. Increased deposition of C3b and cell lysis occurred only upon addition of divalent antibody (57).

Although ACP-mediated, antibody-dependent lysis of infected cells has now been shown to occur in several virus systems (45), studies by others suggest that antibody may not always be necessary. Evidence obtained with Rous sarcoma virus-transformed quail tumor cells has suggested that the ACP, in the absence of detectable antibody, can cause the lysis of Rous sarcoma virus-transformed cells. The dependency of the ACP was demonstrated by showing that the serum reactivity was heat labile, required magnesium, and was not present in zymosan- or inulin-treated sera (68). More rigorous studies will be necessary to prove the antibody-independent nature of this phenomenon.

More recently, another complement-mediated lytic mechanism has been described *in vitro*. Bovine polymorphonuclear leukocytes (PMN) have been shown to mediate, in the presence of complement and in the absence of detectable antibody, lysis of herpesvirus-infected bovine kidney cells. PMN were much more effective in lysing target cells than were macrophages or lymphocytes (19). Complement-facilitated, antibody-dependent cellular cytotoxicity was originally thought to be the mechanism of this phenomenon (54); however, PMN from antibody-positive animals were subsequently shown to be no more effective than PMN from nonimmune animals (19). The mechanism of this complement-mediated cytotoxic phenomenon is not known, but it is possible, since PMN contact with target cells is required for lysis, that the virus-infected cells activate complement and facilitate the binding of PMN, by virtue of their C3b receptors, to the infected cells.

ROLE OF COMPLEMENT IN VIRAL INFECTIONS *IN VIVO*

Recent studies suggest that the several mechanisms discussed above by which the complement system can interact with virus or virus-

infected cells *in vitro* may be of importance *in vivo* during viral infections. Both beneficial and immunopathological roles for complement have been described in a limited number of animal models.

The first demonstration for a role of complement *in vivo* in the host response to virus infection was shown by Oldstone and Dixon (42). In these studies, the susceptibility of different mouse strains to LCMV infection was studied. It was shown that B10.D2 old-line mice, which lack serum C5, were more resistant to challenge with LCMV than the congenic, C5 normal, B10.D2 new-line mice. Since C5-negative mice were more resistant to LCMV challenge than C5-positive mice, these early studies suggested that terminal complement components may play an immunopathological role in virus infections. Mice of other strains tested which were C5 negative (e.g., SWR/J) were highly susceptible, but since other genetic factors may also influence susceptibility to LCMV, conclusions like those made in the congenic B10.D2 mouse system may not be valid. However, it is interesting to note that the levels of virus in the blood, brain, kidneys, and livers of 3-month-old C5-negative mice (B10.D2 old line and SWR/J) persistently infected with LCMV at birth were higher than those in the organs of C5-positive mice (C3H/HeJ) (42). Thus, these early studies suggested that complement may play a dual role in the host response to viral infection. Further studies performed with the LCMV model showed that mice depleted of serum hemolytic complement levels by treatment with cobra venom factor (COVF is cobra C3b, is insensitive to mammalian I, and causes temporary depletion of serum C3 levels) were more resistant to LCMV challenge (44). These studies further suggested that complement played an immunopathological role in the normal animal. Complement was also implicated in the pathology of LCMV-induced glomerulonephritis, as it was demonstrated that complement, virus, and antibody were deposited in glomerular-capillary basement membrane (7, 44).

Recent studies have shown that the complement system plays an important role in the host response to viral infection (Table 3). Two studies showed that, in mice depleted of complement by COVF treatment, more severe infections with Sindbis virus (22) or influenza virus (21) occurred. In the influenza virus model, B10.D2 old-line and new-line mice were also studied, and in B10.D2 old-line mice, as well as in the COVF-treated mice, greater amounts of influenza virus were present in the lungs after infection and a higher mortality rate was observed (21). In the case of Sindbis virus infection of mice, three important observations were made (22). Firstly,

TABLE 3. Studies on the role of complement in viral infections of mice

Virus	Effect of complement depletion	Reference
LCMV	Dose of virus causing 50% mortality increased	44
Influenza A	Increased levels of virus in lung and increased mortality	21
Sindbis	Prolonged viremia; increased levels of virus in CNS; later deaths	22, 23
Rabies	Increased levels of virus in CNS	36

despite the fact that there were no significant differences between COVF and control mice in local replication of virus in muscle after subcutaneous inoculation, the viremia was prolonged in the COVF-treated mice (Fig. 3). Secondly, at the peak of virus replication in the brain, 1,000-fold more virus was present in the brains of the COVF-treated mice. However, despite the 1,000-fold greater levels of virus in the central nervous system (CNS) of treated mice, overall mortality (30%) was the same in both groups. Thirdly, the COVF-treated animals died significantly later (approximately 2 days) than control infected mice. The later deaths coincided with the return of normal serum C3 levels in the previously de complemented mice. Thus, these studies suggested a dual role for complement in Sindbis virus infection of mice. Early in the infection, during the period of viremia when there is not yet any detectable antibody, complement in some manner controls viremia. Thus, in the COVF-treated animals, viremia persists and makes infection of target tissues (in this case the CNS) a more likely event. This could account for the 1,000-fold greater levels of virus in the brain of the treated animals. The later deaths in the COVF-treated mice may indicate that the complement system in normal mice plays an immunopathological role in the normal animal by killing virus-infected cells in the brain, elaborating chemotactic factors, or promoting the mononuclear inflammatory response or by a combination of these. In the case of COVF-treated mice, when C3 levels return to normal these immunopathological events are manifested by later deaths.

Further studies were performed to determine whether the major difference seen in the pathogenesis of Sindbis virus infection in normal and complement-depleted mice (i.e., 1,000-fold increase in brain virus titers) was due to a lack of complement-mediated effector mechanisms operative in the CNS in the COVF-treated mice or to a lack of effective complement interaction with blood-borne virus early in the infection. In

studies designed to determine whether complement depletion directly influenced the replication of Sindbis virus in the CNS, animals were depleted of complement by COVF treatment and were inoculated intracerebrally with two strains of Sindbis virus which varied in their virulence (23). As observed for subcutaneously inoculated animals (22), there was no difference in percent mortality after intracerebral inoculation of Sindbis virus in complement-depleted animals and control mice. However, unlike the results observed after subcutaneous inoculation, there were no differences in levels of virus in the CNS after intracerebral inoculation (23). In other experiments, animals were inoculated subcutaneously; however, COVF was not administered until after the viremia had ended. These studies also showed no differences in the growth of the virus in the CNS between control and COVF-treated animals and therefore suggest that complement-mediated defense mechanisms operate during the period of viremia to limit subsequent infection of the CNS (Fig. 4). Direct investigations were performed to determine whether the complement system interacted with virus and influenced viremia. It was shown for two strains of Sindbis virus that, in mice depleted of complement by COVF treatment, a clearance defect was evident after intracardiac inoculation of virus. Thus, these studies suggested that the complement system interacts with virus, during the viremia, and influences the clearance of virus from, or the inactivation of virus in, the blood stream in the absence of antibody (23). The mechanism(s) by which the complement system accomplishes this has not yet been resolved. However, as mentioned above, we have shown that Sindbis virus can activate both the ACP and the classical complement pathway in the absence of detectable antibody (25); thus, the complement system may neutralize virus or deposit C3b on the virion surface and influence its uptake by the reticuloendothelial cell system.

Further studies in the Sindbis virus model have shown the importance of the terminal complement components (C5 through C9) in recovery from infection. Infection of 2-week-old B10.D2 old-line mice with Sindbis virus resulted in approximately 80% mortality, whereas only 20% of B10.D2 new-line mice succumbed to fatal Sindbis virus infection. There were no differences in the viremia in these mice which, unlike COVF-treated mice, possess normal C3 levels; however, there were significantly greater amounts of virus present in the brains of B10.D2 old-line mice 5 days after infection (24). These studies suggested that there may have been a previously undetected role for complement in limiting virus growth in the CNS itself when seeding of the brain (viremia) is held constant

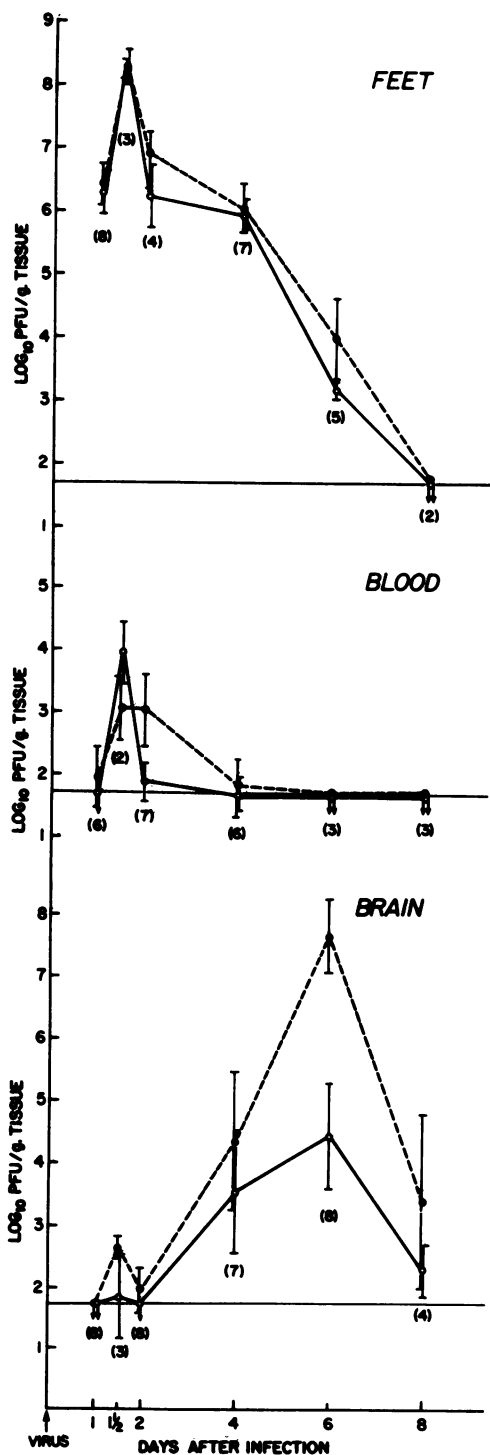


FIG. 3. Levels of Sindbis virus in feet, bloods, and brains of 12-day-old normal (—) and COVF-treated (---) mice inoculated subcutaneously with virus. A prolonged viremia and greater levels of virus were noted in the brains of treated mice. Numbers in

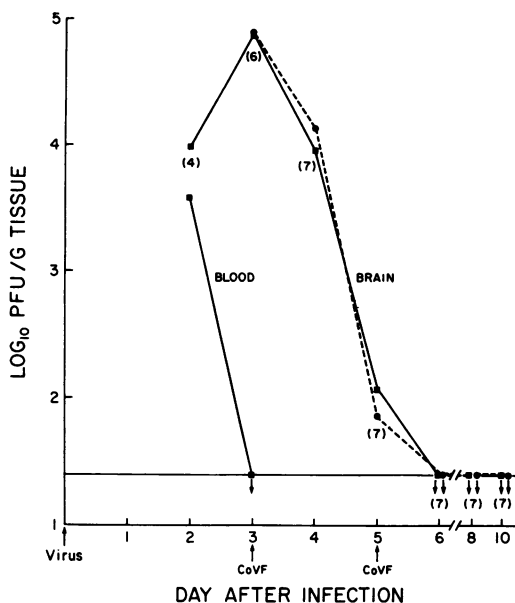


FIG. 4. Levels of Sindbis virus in brains of normal (—) and COVF-treated mice (---) mice after subcutaneous inoculation of virus. When COVF is given after the viremia has ended, no differences in level of virus in the brain are observed. Numbers in parentheses indicate numbers of animals in each group assessed at the indicated times. (Reprinted from reference 23 with permission from the University of Chicago Press, Chicago, Ill.)

and contributions of the terminal complement components are definitively eliminated. Thus, these studies implicate either the generation of chemotactic factors (C5a) or the terminal lytic components of complement (C6 through C9) in controlling some aspects of virus infection.

One other *in vivo* study has implicated the complement system as being important in viral infections. Mice treated with COVF and inoculated intracerebrally with rabies virus demonstrated delayed clearance of virus from the CNS (36). These results are comparable to those reported for Sindbis virus infection in C5-deficient mice.

ROLE OF THE COMPLEMENT SYSTEM IN VIRAL INFECTIONS OF HUMANS

Although it has been clearly demonstrated in animal models that the complement system can play both beneficial and immunopathological roles in the host response to viral infection, limited data are available concerning the role of

parentheses indicate number of animals in each group assessed at the indicated times. (Reprinted from reference 22 with permission from the Williams & Wilkins Co., Baltimore, Md.)

complement in viral infections of humans. There are two major reasons for this lack of information. Firstly, although patients with inherited deficiencies of complement components are described in the literature, these patients usually present with recurrent or chronic bacterial infections, and problems with viral infections are not usually described. Secondly, there are only limited studies which can be done in humans that relate to the role of complement in the pathogenesis of infectious diseases.

Most investigations on the role of the complement system in viral infections of humans have concentrated on changes in serum complement profiles during infection. There is a major problem with interpretation of these studies. When alterations of serum complement components are found, it is difficult to discriminate between consumption of complement components, activation of complement by virus-antibody complexes, changes in synthesis or catabolism of complement components, or extravasation of complement components as a result of the virus infection. Despite these limitations, some useful information has been collected.

In a group of 50 children with uncomplicated acute measles virus infections, it was found that 48% of patients had reduced serum C1q levels, 12% had reduced C4 levels, 26% had reduced C3 levels, and 16% had reduced C5 levels as measured immunochemically by radial immunodiffusion. Ten of the patients showed complement profiles indicative of activation of the classical pathway (decreased C1q, C4, and C3 levels), whereas seven showed decreased C3 levels with normal C4 and C1q levels, which the authors suggested as evidence of activation of the ACP (9). However, factor B and C7 levels in these patients were normal, suggesting that the ACP and terminal complement components were not utilized. The decreased C3 levels, therefore, in patients not showing decreased C1q and C4 levels may indicate an effect of measles virus, known to infect mononuclear cells, on C3 synthesis in these patients. Eleven of the patients demonstrated reduced levels of serum C1q only. Despite this pattern, immune complexes, which would consume C1, were not detected by a C1q precipitation test (9).

Charlesworth and colleagues have also studied serum complement profiles in 34 patients with infectious mononucleosis (8). When the values for all of the patients were pooled, mean values for all complement components measured (C1q, C1s, C1 inhibitor, C4, C3, factor B, properdin, C5, and C7) were not significantly different from values in normal patients. However, in three patients with complicated cases (one severe hemolytic anemia, one severe arthralgialgia, and one proliferative glomerulonephri-

tis), reduced C3 levels were observed. Two had decreased C1q and C4 as well as reduction in the terminal complement components C5 and C7. The patient with glomerulonephritis had markedly reduced C3 levels and was positive for C3NeF but had normal C1q and C4 levels. In these complicated cases the decreased levels of C3 may be related to the presence of C3NeF (shown to be increased in these studies) and its ability to enhance activation of the ACP (C3NeF inhibits the ability of H and I to interact with factor B and C3 on the surface of activating particles). However, serum properdin levels were not reported for these three patients with complications, and this detail would be helpful in determining the mechanism of the decreased C3 levels. In the uncomplicated cases, complement levels may be influenced by direct infection of macrophages by Epstein-Barr virus, the causative agent of infectious mononucleosis. Thus, the reported decreases in C4 and C3 could be due to a direct inhibitory effect of the infection on synthesis of complement components.

Two studies have examined complement levels during acute viral hepatitis infections of children. In one study decreased hemolytic C3 and C4 levels were found in the acute phase of disease, with levels returning to normal during convalescence (1). Since peak immune complex levels were also detected at the time of decreased C3 and C4 levels, these studies suggest activation of the classical pathway, with the reservation that liver damage may have had a major influence on serum levels of C3 and C4. In a second study of acute viral hepatitis, decreased levels of hemolytic C1, C4, C2, C3, and C5 were observed as well as decreased levels of immunochemically reactive C1s, C4, factor B, and C3. C1q levels were normal (15). In one patient, who received infusion of fresh plasma containing normal levels of complement components, C4 levels were followed relative to another patient, with α -1-antitrypsin deficiency, who also received fresh plasma. Within 8 h after plasma transfusion, there was no detectable C4 in the serum of the hepatitis patient, whereas C4 levels in the control patient remained at 30 to 40% up to 70 h after transfusion. These studies also showed decreased prothrombin levels in the hepatitis patient, suggesting that, in part, the decreased complement levels were related to liver damage. However, there was also rapid catabolism of C4 and C3 in patients receiving fresh plasma, suggesting that complement consumption as well as decreased synthesis contributed to the depressed complement levels. It was also noted that low levels of complement persisted in patients who died, probably reflecting severe liver damage (15).

In a study of the complement system in pa-

tients infected with Junin virus, an arenavirus, and presenting with Argentine hemorrhagic fever, total hemolytic complement levels were reduced by 68% in 10 patients with moderate or severe disease (13). In these patients C2, C3, and C5 levels, as determined by immunochemical techniques, were 12 to 60% below normal, whereas C4 levels were 160% of normal levels. Unlike the mouse model of an arenavirus (LCMV) infection discussed previously (43), these results do not support the involvement of immune complexes in the pathogenesis of Argentine hemorrhagic fever (no decreased C1 or C4), but complement activation may play a role in some other respect.

All of the studies outlined above on the effect of viral infections on human complement profiles were performed on patients who were infected with viruses that either infect mononuclear cells (e.g., measles, Epstein-Barr virus) or have cytopathic effects on hepatic cells (e.g., hepatitis virus). Thus, it is not possible to delineate with any certainty from these studies the role that complement plays in viral infections of humans. There are, however, a few suggestive notes which have been made in case reports of patients with inherited deficiencies of particular complement components. For example, in the study of kindred in which a member had a complete C3 deficiency, the brother of the C3-deficient patient, the son of two heterozygotes, died at 21 months of age with documented St. Louis encephalitis (47). Although the death occurred prior to the recognition of the C3-deficient patient, it is tempting to speculate that the brother who died from viral encephalitis may also have been C3 deficient. Similarly, the findings at autopsy in a patient with an inherited deficiency of C4 and systemic lupus erythematosus showed intranuclear inclusion bodies in the lung, and cytomegalovirus was isolated from both liver and lungs (28).

Another interesting association exists between systemic lupus erythematosus, complement deficiencies, and C-type viruses (retroviruses). An association between systemic lupus erythematosus and deficiency of the early complement components has been observed. Furthermore, expression of C-type viruses has been reported in kidney tissue from systemic lupus erythematosus patients (48). Since C-type viruses are lysed by the action of the classical pathway (65), it is conceivable that a deficiency in any of the early complement components could increase the yet unknown risks of C-type virus expression in humans.

SUMMARY

Many lines of evidence indicate that the complement system plays an important role in the

pathogenesis of viral infections. Studies *in vitro* have shown that complement, in either the presence or the absence of specific antibody, can neutralize virus by one or more mechanisms. Furthermore, both the classical pathway and the ACP have been shown to participate in virus neutralization. In addition, the ACP can cause lysis of antibody-coated virus-infected cells, suggesting that, *in vivo*, complement may act not only on free virus but also on infected cells. Studies in animal models have demonstrated that complement does in fact play a significant role in viral infections and that the action of the complement system may be beneficial or harmful. Although the mechanisms by which the complement system exerts influence *in vivo* are unknown, studies have demonstrated that complement either independently or in the presence of antibody can exert beneficial effects at sites of local viral replication or during the period of viremia. Furthermore, the presence of complement-containing immune complexes in tissue, and the delayed death and increased doses of virus required for lethal infection in complement-depleted animals, suggest that the complement system can also exert immunopathological effects during viral infections.

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