Complement Effector Mechanisms in Health and Disease*

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Complement is an effector system able to mediate a number of biological activities in vitro and in vivo. Most familiar is the ability of the system to mediate the lytic destruction of numerous kinds of cells and pathogenic organisms including bacteria, viruses, and virus-infected cells. In addition, the complement system also activates neutrophils, monocytes, basophils, mast cells, and lymphocytes to perform specialized functions. While generally considered to be confined to the effector side of immune reactions, recent evidence indicates that the complement system also directly recognizes and is triggered by a number of bacteria and viruses as well as virus-infected cells in the absence of antibody. In such reactions, complement fulfills the recognition role normally associated with the antibody molecule or immune lymphocyte. The complement system may thus also function as a natural surveillance system operative prior to the induction of specific immunity.

Involvement of the complement system in biological reactions has been ascertained by several techniques over the years. These include quantitation of individual complement components in human sera and demonstration of complement deposition in diseased tissues in human diseases and in experimental diseases in animals. Such techniques, however, have limitations in specificity and sensitivity. Assays which detect specific features of the complement activation process have become available in recent years. These tests detect the physical, chemical, or antigenic changes characteristic of the complement activation process. These assays are extremely specific and quantitative; furthermore, most are usable with samples from patients. Three general approaches have been utilized to develop such specific quantitative assays for complement activation. The first includes assays which quantitate activation-specific limited proteolysis of the complement components. The second type of assay includes tests which detect and quantitate new antigens or other activation-specific antigenic changes. The third category is represented by assays which detect and quantitate the protein-protein complexes characteristic of the activation process. Examples of tests presenting each of these approaches are given.

Data from the above approaches together with studies of in vitro participation and functions of complement molecules in selected reactions such as opsonization, phagocytosis, chemotaxis, smooth muscle contraction, mediator release, etc., indicate that the two most important biological functions of complement are first, the production of an acute inflammatory response and second, the destruction of infectious agents. Acute inflammatory responses serve to confine infective processes and thus dissemination. The second major in vivo function pertains to the role of the complement system in the control of infection. The complement system directly aids in the destruction of bacteria, viruses, and virus-infected cells by several mechanisms, and facilitates interactions with effector cells leading to either phagocytosis or extracellular destruction of pathogens. These processes and their interrelationships are described.

The primary functions of the immune system include the destruction, neutralization, and elimination of pathogenic agents and altered host cells. Viruses and bacteria represent the majority of human pathogenic agents. As replicating agents, they have the ability to serve as a persistent or increasing antigenic challenge, furthermore, through mutation and other devices, they have the ability to elude host defenses. It is likely that they are largely responsible for driving the immune system to ever greater complexity and diversity through evolution for not only must the immune system recognize changing structures on bacteria, viruses, and virus-infected cells with a high degree of specificity, but it must differentiate these antigens from host structures. The immune system is conventionally divided into humoral and cellular arms with the humoral category including the actions of antibody and complement and the cellular arm the actions of lymphocytes and various other effector cells. These distinctions are artificial as the systems are mutually interdependent and function synergistically.

Complement, the focus of this review, is an effector system capable of mediating a number of biological activities in vitro and in vivo (Fig 1). Most familiar is the ability of the system to mediate the lytic destruction of numerous kinds of pathogenic organisms including bacteria, viruses, and virus-infected cells. Separate from such direct effects, the complement system also triggers or activates various effector cells, including neutrophils, monocytes, basophils, mast cells, and lymphocytes to perform various functions. Depending on the effector cell involved, complement may trigger the release of secondary mediators such as histamine or products of arachidonic acid metabolism, or it may activate intracellular processes, initiate directed motion, facilitate phagocytosis, and extracellular destruction of pathogens as well as modulate immune reactions of several kinds. While generally considered to be confined to the effector side of immune reactions, recent evidence indicates that the complement system also recognizes and is triggered by a number of bacteria and viruses as well as virus-infected cells in the absence of antibody. In such reactions, complement fulfills the recognition role normally associated with the antibody molecule or immune lymphocyte. The complement system may thus also function as a natural surveillance system operative prior to the induction of specific immunity.

CONSTITUENTS AND REACTION MECHANISMS

The constituents of the complement system, including the various regulator proteins, are 20 immunologically distinct plasma proteins [2-4]. These proteins together comprise approximately 15% (w/w) of the globulin fraction of plasma as their cumulative concentration exceeds 300 mg/dl. The complement proteins circulate as individual entities, but following activation, which is a prerequisite for the manifestation of all of the functional activities of the complement system, the individual factors or components undergo a series of precisely...
regulated interactions with the activating substance and with each other. Activation is not a single event, but rather a dynamic continuing process which enables the proteins of the system to become interacting members of a functionally integrated system.

The reactions of the complement components and factors with the activator and with each other can be grouped into several reaction units, each of which involves several complement proteins (Fig 1). There are two entirely separate activation pathways termed the classical [5,6] and the alternative or properdin pathways [7,8] respectively. These activation pathways are independently triggered by certain types of agents. For the classical pathway these include immune complexes containing IgG1, IgG2 or IgG3, or IgM antibodies. In addition, however, the classical pathway may be activated, in the complete absence of antibody, by a number of different kinds of substances, all of which have pathobiological potential in humans. These include several kinds of bacteria, viruses, and virus-infected cells, lipid A and rough lipopolysaccharide molecules, membranes of transformed and altered cells, lysosomal enzymes, and a number of other substances of diverse chemical and physical composition [9-11].

During the activation process, the first component of the classical pathway, termed C1, is converted from its native form into a proteolytic enzyme which activates the next two reacting complement components, C4 and C2, by limited proteolysis [5,6,9,10] (Fig 1). The larger cleavage fragments of each of these molecules have the ability to bind to each other and form a C4,2 protein-protein complex. The C4,2 complex represents another de novo-formed complement enzyme which has the ability to proteolyze C3, the next reacting factor. With the cleavage of C3, again by hydrolysis of a single peptide bond, yet another enzyme is formed which has the ability to cleave and activate C5 and thereby to initiate the terminal portion of the complement reaction sequence (Fig 1). The classical pathway thus consists of a series of enzyme-substrate reactions in which factors are activated by limited proteolysis. The larger cleavage fragments interact with each other thereby forming protein-protein complexes which have de novo enzymatic activity directed at the next reacting complement factor. During classical pathway activation, C1 binds to the activator whether it be an immune complex, bacterium, virus, virus-infected cell or other activator. Although a relatively minor proportion of the larger cleavage fragment of C4, C2 and C3 bind to the surface of the activator, and to each other, assembly of the complement enzymes on activator surfaces is far more efficient than in the fluid phase. Reactions on the activator surface also obviously focus the actions of the complement system on the activator. While the smaller cleavage products do not generally bind to the surface of the activator and are released into the fluid phase, some of these fragments have important biological activities, as will be considered subsequently.

The alternative complement pathway is usually activated directly, in the absence of antibody [7,8]. Activators include complex polysaccharides, particularly those associated with bacteria, certain viruses and virus-infected cells and other types of altered cells as well as lipopolysaccharide molecules. Specific antibody also potentiates a number of alternative pathway activation reactions and appears to be, in certain limited instances, also responsible for activation. The alternative pathway is initiated by the binding of an altered form of C3 or the larger cleavage product of C3 to the surface of activators (Fig 1). On such structures, it interacts with Factor B, which after complexing is rendered susceptible to limited proteolysis by Factor D, a proteolytic enzyme. The C3,B protein-protein complex containing the larger cleavage products of C3 and Factor B represents a de novo proteolytic enzyme able to fragment additional molecules of C3, again by limited proteolysis. The attachment of the larger cleavage product of C3 in proximity to the C3,B complex forms another proteolytic enzyme, C3a,B able to hydrolyze C5 and initiate the membrane attack pathway. Thus, and in analogy to the classical pathway, activation involves the sequential formation of protein-protein complexes with de novo enzyme activity.

The membrane attack pathway is initiated following limited proteolysis of C5 by the C5 cleaving enzyme of either the classical or the alternative complement pathways (Fig 1) [12, 13]. Following cleavage of C5, the remaining components, C6, C7, C8, and C9 interact with C5 and with each other to form a large multimolecular complex, C5b-9, termed the membrane attack complex. This protein-protein complex has the transient ability to disrupt lipid-containing membranes. It is responsible for the cytolytic activity of the activated complement system.

The activities of the complement pathways are precisely regulated by a number of processes including lability of binding sites, dissociation of complexes and by the actions of a group of proteins, enzymes, and functional site inhibitors. With regard to the latter, the classical pathway is modulated by the actions of C1-inhibitor, a protein which regulates the actions of activated C1, and by C4-binding protein, which binds to the larger cleavage fragment of C4 and facilitates the further degradation of this fragment by a complement regulator enzyme termed Factor I. The alternative pathway is up-regulated by the action of properdin, a protein which stabilizes the actions

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**Fig 1.** Pathways and biology of the complement system. Reprinted from [1] with permission.
of the C3B and C3nB enzymes and down-regulated by a protein termed Factor H which binds to the larger cleavage fragment of C3 and facilitates its destruction by Factor I. Another regulator, S protein, controls and modulates the activity of the assembling membrane attack complex. Finally, a carboxypeptidase in plasma termed carboxypeptidase N or anaphylatoxin inactivator removes the C terminal arginine residues from C3a, C4a, and C5a, the smaller cleavage fragments of C3, C4, and C5, respectively, thereby abrogating certain of the biological activities of these fragments [14].

ASSESSMENT OF COMPLEMENT INVOLVEMENT

Involvement of the complement system in biological reactions has been ascertained by a number of techniques over the years [2,15–17]. These include quantitation of individual complement components in human serum either by functional assays based on immune hemolysis or by immunocchemical methods, such as the Mancini and rocket immunodiffusion techniques. Reduction in the levels of certain complement components in the circulation is strongly suggestive of in vivo complement activation. This is in part because the functional activity of many of the complement components is lost with activation; furthermore, several of the activated, cleaved complement molecules, in particular, the larger cleavage products of C3, C4, C5, and Factor B, are rapidly cleared from the circulation. The most useful measurements, regardless of the method used, are of C2, C3, C4, C5, and Factor B. Selective activation of either the classical or alternative pathways may be ascertained if multiple components are measured since a reduction in the levels of C2, C3, and C4 in the circulation without marked changes in the concentration of Factor B strongly suggests classical pathway activation while a reduction in Factor B and C3 levels with minor changes in the classical pathway component levels is characteristic of alternative pathway activation. Predominant activation of the classical pathway characterizes several infectious diseases and autoimmune diseases, particularly those involving immune complexes. Alternative pathway activation is found in membrano-proliferative glomerulonephritis, certain infectious diseases, and in shock accompanying burns, traumatic injury, and infection with gram negative bacteria.

A major limitation of measurements of the circulating levels of complement components as a technique for assessing complement activation is that only very marked activation can be detected by these techniques. This is because the complement factors are normally synthesized and metabolized at approximately 2% of the plasma pool per h, an extraordinarily rapid rate. Within this normal background of rapid synthesis and degradation, increased utilization is difficult to detect.

Complement involvement has also been frequently demonstrated by showing deposition of complement components, generally C1q, C3, and C4 and sometimes properdin, Factor B, or C5b-9, often together with immunoglobulin, in areas of tissue damage in human diseases and in experimental diseases in animals. Most often, the bound complement components are demonstrated by immunofluorescence techniques. While the finding of complement components in tissues, particularly in association with a pathogen or immunoglobulins constitutes excellent evidence for complement involvement, the extent of activation cannot be quantitated, and temporal correlations are not possible since complement components can remain in tissues for prolonged periods of time.

A number of assays which detect specific features of the complement activation process have become available in recent years [15]. These tests detect the physical, chemical, or antigenic changes which occur in the complement components with the activation process. Assays designed to detect and/or quantitate such activation-dependent changes are extremely specific and most allow quantitation of activation; furthermore, many of the tests are applicable to use with samples of blood or tissues from patients.

The three general approaches which have been used are depicted in Table I. The first includes assays which detect the limited proteolysis of certain of the complement components characteristic of the activation process. The second approach includes assays which detect and quantitate either new antigens or other antigenic changes appearing as a result of activation. The third approach is represented by tests which detect and quantitate the protein-protein complexes which occur during the activation process.

An example of an assay which detects cleavage of a complement component during activation is depicted in Fig 2. With activation of the classical pathway, the C1r and C1s subunits of Cl undergo limited proteolysis. This is shown in Fig 2, which depicts these changes in C1s, as seen in a sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis [15,18]. During C1 activation, the 87,000 dalton polypeptide chain of native C1s is cleaved at a single residue yielding 59,000 and 28,000 dalton fragments. Cleavage can be readily assessed by tracking radioactivity. Similar studies can be carried out with a number of the other complement components which undergo limited proteolysis during the activation process. Several other approaches which detect specific activation-dependent cleavage of the complement components during the activation process are shown in Table I. Notable among these are the very sensitive, recently developed radioimmunoassays which detect and quantitate the smaller C3a, C4a, and C5a fragments in C3, C4 and C5 respectively [14,19–21]. These radioimmunoassays, which are now commercially available, detect less than 20 ng/ml of the activated products in human plasma or sera. They are thus far more sensitive than other approaches to measure C3, C4, and C5 cleavage. Furthermore, they are quantitative. Such assays furnish some of the best of the newer quantitative techniques to assess complement activation by measuring specific features occurring during the activation of the complement system.

The second type of assay system detects activation-induced changes in the antigenic structure of the complement components (Table I). In some cases new antigens not present in the native molecules are produced during activation. Antibodies formed against these neoantigens do not react with native molecules. Neoantigens are known to appear with activation of C1q, C3, Factor B, and the C5-C9 membrane attack complex;

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it is likely that more such antigens will be described with additional applications of the monoclonal antibody technology to the study of complement activation. An assay of this type which has been employed is the Clr-C1s antigenic ratio [22]. With activation of the complement system in serum, Clr antigenicity disappears. This dramatic change is due to masking of many of the major antigens of activated Clr by the control protein C1-inhibitor which rapidly binds to activated Clr [23]. This disappearance of Clr antigenicity with activation has been utilized to demonstrate activation in patient samples [22–24]. A related approach has been employed to determine the functional levels of C1 inhibitor in human serum or plasma and has been proposed as a diagnostic test for C1-inhibitor functional deficiency as associated with certain kinds of angioedema i.e., hereditary angioedema (HAE) [25]. In this assay, shown in Fig 3, a known complement activator such as aggregated immunoglobulin is incubated with normal serum as well as with the test serum suspected of having reduced levels of C1-inhibitor. Clr is subsequently assayed by radial immunodiffusion. In normal serum, Clr antigenicity disappears following activation of the complement system, whereas in serum lacking functional C1-inhibitor, there is no reduction in the apparent Clr concentration (Fig 3).

The third type of approach which detects specific features of the complement activation process utilizes assays which quantitate the protein-protein complexes characteristic of the complement activation process (Table I). Since such protein-protein complexes are not present in native serum or plasma but only occur following activation, such assay systems have very high specificity and low background or nonspecific reactivity. Fig 4 depicts enzyme-linked immunosorbent assays which detect protein-protein complexes characteristic of activation of both the classical and the alternative complement pathways [15,25,27]. The classical pathway ELISA detects the complex of C1-inhibitor with activated Clr and C1s, subunits of C1. C1-inhibitor rapidly and irreversibly combines with activated Clr and C1s; it does not interact with the native forms of these molecules and thus the complex is not found in normal serum. The assay is able to detect activation of less than 1% of the classical pathway in human plasma [15,27]. In the assay, antibody to C1s is attached to the plastic microtiter well, the mixture containing activated C1 or a sample from patients potentially containing activated C1 is then added to the wells. Next, enzyme-labeled antibody to C1-inhibitor and finally substrate is added. Color development is proportional to the concentration of the ClrC1s-C1-In complex. Precise quantitation can be afforded by simultaneous inclusion of standards.

The alternative pathway ELISA, shown in Fig 4, detects a complex formed between properdin and C3b [15,26]. This complex is diagnostic of alternative pathway activation. In the test system, antibody to properdin is coupled to the plastic plate, a sample or serum from patients or an activator mixture is added and C3,B complexes are detected with enzyme-labeled antibody to C3. This assay is extremely sensitive and able to quantitate activation of approximately 10 ng/ml of C3 which, in human
plasma, corresponds to activation of only 0.015% of the C3 in plasma.

**BIOLOGICAL FUNCTIONS OF COMPLEMENT IN VIVO**

Information on the biological importance of the complement system has come from two main sources. These are demonstrations of complement involvement by direct measurement or detection in damaged tissues in human diseases and in experimentally-induced diseases in animals employing the kinds of techniques listed above and in Table I. The second source of information has been the study of humans who are congenitally deficient in complement proteins or in complement regulators. As indicated in Table II, such individuals exhibit 1 of 4 types of disease. Genetic absence of components of the classical pathway including the C1 subcomponents, as well as C4 and C2, is frequently associated with autoimmune-type diseases including systemic lupus erythematosus, glomerulonephritis, and occasionally repeated infections. More than half of the 80 or so reported individuals with these deficiencies are clinically ill and suffer from one of these diseases. In contrast, individuals lacking C3 or its regulator protein, Factor I, suffer from recurrent life-threatening infections with pyogenic organisms. The third disease complex characterizes deficiencies of the late reacting complement components, C5, C6, C7, and C8. Recurrent life-threatening systemic infections with gonococci or meningococci occur in more than half of the individuals with these symptoms; systemic infection with *Neisseria* is frequently the presenting symptom of patients with such deficiencies. Patients having congenital deficiencies of C1 inhibitor have hereditary angioedema. Individuals lacking this crucial control protein have uncontrolled activation of the classical complement pathway and also exhibit in vivo activation of the other effector systems regulated by the inhibitor.

Data from the above approaches as well as studies of in vitro participation and functions of complement molecules in selected reactions such as opsonization, phagocytosis, chemotaxis, smooth muscle contraction, mediator release, etc., indicate that the two most important biological functions of complement are the production of an acute inflammatory response and the destruction of pathogens including bacteria, viruses, and virus infected cells (Table III). The complement system has the ability to produce an acute inflammatory response (Fig 1). The inflammatory response is primarily produced by C5a, C4a, and C5a. These hormone-like peptides enhance vascular permeability, release mediators such as histamine from mast cells and basophils, induce smooth muscle contraction, and trigger lysosomal enzyme release from granulocytes. In addition, C5a releases leukotrienes from pulmonary tissue [13,27]. Furthermore, C5a also is chemotactic and able to initiate the directed migration of leukocytes into an area of complement activation. This same peptide also aggregates polymorphonuclear leukocytes and induces additional release of intracellular enzymes and proteins from these cells. The actions of the anaphylatoxins and the secondary mediators released from the cells by the anaphylatoxins are integrated with each other; furthermore, several have similar biological activities and they are mutually self-reinforcing (Fig 1). These in vitro biological activities are responsible for the phlogistic activity of the complement system.

An acute inflammatory response serves the major function in vivo, of confining an infective process and retarding its spread throughout the body. At the same time as the acute inflammatory response is occurring, C5a, also having chemotactic properties, is responsible for triggering leukocyte infiltration. Incoming leukocytes become fixed in the area of complement activation through attachment to specific receptor sites located on the larger cleavage products of C3 and C4. Subsequent phagocytosis and/or release of other mediators and lysosomal enzymes facilitates the destruction of the infective agent. Other properties of the activated complement system including its cytolytic ability also facilitate the cytotoxic destruction of pathogens. These various properties will be described below.

The second major function of the complement system in vivo relates to its role in the control of infection. The complement system directly aids in the destruction of infectious agents, primarily bacteria and viruses by several mechanisms. It also possesses the ability to destroy virus-infected cells. Complement facilitates interactions with effector cells leading to either phagocytosis or extracellular destruction.
COMPLEMENT ACTIVATION BY PATHOGENS

Numerous bacterial and viral structures are antigenic or generate the formation of antibody. On subsequent exposure to the pathogen such elicited antibody can mediate complement activation. In addition, however, many bacteria, particularly gram negative bacteria, directly activate complement in the absence of antibody [11,29,30]; this is also true of a number of viruses and virus-infected cells [31,32]. Such direct activation, which has now been observed for a number of different bacterial and viral pathogens as well as with mycoplasma and parasitic organisms, suggests that the complement system fulfills a primary surveillance function entirely analogous to the actions of natural killer cells.

As noted, some bacteria activate the complement system in the absence of antibody; such activation is augmented by antibody. Certain kinds of gram negative bacteria may be killed by the complement system, in some cases in the complete absence of antibody [33], while in most instances antibody is required for killing [30]. As depicted in Fig 5, bacteria treated with isolated complement components of the alternative pathway together with the purified components of the membrane attack complex in the absence of antibody are somewhat larger and have irregularities in surface structure. The killed bacteria, although not lysed, also possess numerous complement-dependent lesions characteristic of the formation of the membrane attack complex on lipid bilayers when examined by transmission electron microscopy. Bacterial lysis, which is accomplished only on addition of lysozyme, and as depicted in Fig 5, leads to complete loss of structure.

Complement activation by viruses also leads to complement component deposition on the external surface of virus regardless of whether activation occurs directly, or is mediated by antibody. In the case of isolated virions, bound complement components may reduce infectivity by one or more of several distinct mechanisms which have been observed with various viruses. These include aggregation which reduces the net number of infectious particles; envelopment which refers to a coating of antibody and/or complement protein which may interfere with the ability of the virus to attach to and infect a potentially susceptible cell; and cytolysis which irreversibly destroys infectivity [31,32]. Although many viruses may be aggregated in vitro by antibody, complement-dependent agglutination leading to neutralization has thus far been observed only with polyoma virus, a nonenveloped DNA-containing virus. Envelopment as a mechanism of neutralization has been found to be the major process by which antibody and/or complement neutralizes most of the viruses thus far examined. Although most and probably all enveloped viruses may be lysed by complement under appropriate circumstances, as shown, for example, in Fig 6, those viruses which have been carefully studied have been found to be fully neutralized prior to the occurrence of lysis [31].

Complement activation by virus infected cells, whether direct or antibody-initiated, leads to complement deposition on the cell surface. Such components may prevent virus maturation, as has been observed for antibody. The virus-infected cell may also be lysed by the complement system [31,36]. Extensive studies have shown lysis of such cells to be mediated by the alternative complement pathway in conjunction with the membrane attack pathway [31,32,36,37]. Although activation does not require the presence of antibody [38], cytolysis destruction of the cells requires the presence of both antibody and the properdin system [37,38]. Obviously, lysis of the virus-infected cell prevents virus maturation.

Undoubtedly very important in virus defense is the ability of the complement system to augment Fe-dependent opsonization, or to mediate complement receptor-dependent interactions with various effector cells bearing receptors for the larger cleavage products of C3 or C4. Numerous cell types including lymphocytes, macrophages, monocytes, and neutrophils bear receptors for one or more activated complement components; of these, receptors for C3 appear to be the most important. C3 or C4 fragments which become attached to the surface of the bacteria, virus, or virus-infected cell as a consequence of antibody-dependent or antibody-independent activation of the complement system may mediate binding of these activators to the various effector cell types bearing C3 or C4 receptors. Such attachment may trigger specialized reactions by the effector cell, depending on the target and effector cell as well as the complement fragments and receptors involved. Such responses may include chemotaxis, extracellular cytotoxicity, phagocytosis, activation of oxidative metabolism, and the respiratory burst, mitogenesis, stimulation of arachidonic acid dependent reactions as well as a number of other reactions [14,39,40].

Bacteria which have reacted with antibody and/or the complement system represent an opsonic stimulus for the polymorphonuclear leukocyte. An example of phagocytosis of complement-coated bacteria by neutrophils is depicted in Fig 7 [40]. In this particular instance, phagocytosis, as shown in the right-hand panel, is independent of involvement of antibody since the purified alternative pathway proteins at physiologic concentrations in the absence of antibody were employed for this study. In many other instances, such phagocytosis is antibody as well as complement-dependent [30,40].

Antibody and/or complement components deposited on isolated virions and on virus-infected cells as a consequence of

Fig 5. Scanning electron microscopy of alternative pathway treated E. coli K12. Shown are E. coli treated at 37°C for 60 min with buffer or the heat-inactivated (56°C, 30 min) isolated component mixture (upper left panel), the isolated component mixture (upper right and lower left panels), or the isolated component mixture containing 10 µg/ml egg white lysozyme (lower right panel). Concomitant analysis of viability and lysis revealed that compared to the control (upper left panel), samples (upper right and lower left) were killed but not lysed, whereas sample (bottom right) was killed and lysed. Reprinted from [33] with permission.
FIG 6. Sections of purified lymphochooriomeningitis virus (LCMV) exposed to (A) normal control serum, (B) heated guinea pig serum containing antibody to LCMV, (C-F) guinea pig serum containing antibody and complement. Arrows in (F) point to disrupted viral envelope releasing viral core material. Reprinted from [35] with permission.

FIG 7. Scanning electron micrograph of polymorphonuclear leukocytes (PMNL). PMNL were incubated for 5 min at 37°C with unopsonized E. coli 04 (A) or with E. coli 04 which had been preincubated with the purified alternative pathway proteins (B). After washing, PMNL were fixed with modified Karnofsky fixative and prepared for electron microscopic analysis. Reprinted from [40] with permission.

Complement activation can mediate the attachment of the virus particles or infected cells to lymphocytes and other cell types bearing such receptors. Such interactions may lead to extracellular destruction of virus-infected cells [36] and potentially also of isolated viruses, although the latter has not been convincingly shown. The bound protein may also be opsonic and trigger ingestion. An example is shown in Fig 8 which depicts phagocytosis of antibody and complement-coated Epstein-Barr virus, a human herpesvirus, by polymorphonuclear leukocytes (GR Nemerow, NR Cooper, unpublished studies).

CONCLUSIONS
This review has briefly summarized the nature of the complement proteins and their reaction mechanisms and described various tests to determine participation of this system in human disease. It has concluded with an assessment of the kinds of biological reactions in which complement is likely to play a major role in vivo. These include mediation of, and involvement in, inflammatory processes. In infectious diseases, inflam-
nervation serves to restrict the spread and dissemination of the infection. Inflammation is also a prominent feature of a number of other diseases which are not infectious in origin. In these instances, such as observed in autoimmune diseases, as well as in tissue injury due to infection, trauma, shock, or burns, complement may both produce and also augment the inflammatory processes which occur. In all of these conditions, complement mediates processes which are destructive to normal tissues. The other major category of complement-dependent actions has to do with the prevention, control, and recovery from infection. The complement system likely finds its major importance in vivo in this broad area.

In addition to these well-characterized actions, other properties which have been incompletely studied suggest that complement fulfills other physiogenic functions in vivo. For example, the reasons for the increased susceptibility of individuals genetically lacking in the components of the classical pathway to autoimmune diseases is completely obscure. This increased prevalence may be related to an as yet unappreciated role for the complement system in immune recognition, regulation, or other related reactions. In this context, the presence of the structural genes for C2, C4, and Factor B, but not other complement components within the major histocompatibility complex in humans, a region involved in self, nonself discrimination, may be important. Another potentially related, and as yet not understood role for complement may be in the induction of immune responses to certain T-dependent antigens. A number of papers have been published which have suggested a role in complement for a number of kinds of reactions involving various aspects of immune regulation. It is likely that further study will reveal additional biological functions for this highly complex, well-differentiated system.

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