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Review

# Complement and innate immunity

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## 1. Innate immunity

Innate immunity is the first line of host defense for multi-cellular organisms. In contrast with adaptive immunity, which is restricted to vertebrate animals, innate immunity is more ancient and is used by invertebrates such as insects and echinoderms, as well as by higher animals. Whereas adaptive immunity involves receptors that are generated by somatic mechanisms and relies on clonal expansion of subsets of T- and B-lymphocytes bearing these receptors, innate immunity uses fixed, germ line-encoded mechanisms that are acquired through natural selection during the course of evolution (Hoffmann et al., 1999).

Innate immunity is generally regarded as a less specific and unsophisticated mechanism of host defense, which plays a more important role in primitive life forms. In higher animals, innate immunity was once viewed as an evolutionary relic whose functions had largely been superseded by the advent of adaptive immunity. Accordingly, until recently, much greater emphasis has been placed on the study of adaptive immunity than on innate immunity in mammalian systems. In the last few years, however, there has been a resurgence of interest in innate immunity, and a new view is emerging as we begin to achieve a deeper understanding of this ancient form of immunity (Fearon and Locksley, 1996; Bendelac and Fearon, 1997; Carroll and Janeway, 1999; Hoffmann et al., 1999).

The convergence of studies from different directions and approaches has revealed a remarkable similarity in the molecular mechanisms and signal transduction pathways of innate immunity among divergent life forms, ranging from those lacking adaptive immunity (such as insects and plants) to those with highly developed adaptive immunity (such as mammals) (Hoffmann et al., 1999; Borregaard et al., 2000). Furthermore, it is now widely recognized that innate immunity, in addition to providing the first line of defense in higher animals, also plays a critical role in priming and instructing the adaptive immune response (Fearon, 1997; Medzhitov and Janeway, 1997a). The complement system is composed of a series of plasma proteins and represents an important arm of the innate immune system in both invertebrate and vertebrate animals. In this review, we will summarize recent progress in the complement field, which highlights the role of complement as an effector system of innate immunity in the traditional sense

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as well as the reciprocal interplay between complement and the adaptive immune response.

## 2. Innate immunity consists of multiple effectors including complement

Broadly speaking, innate immunity encompasses a wide range of passive and active mechanisms of constitutive host defense against infection. Among them are physical barriers, such as the skin and mucosal surfaces of the digestive and respiratory systems, and low pH levels in the stomach. Two major components of active innate immunity are the body's phagocytic cells, such as neutrophils and macrophages, and natural killer cells. Both neutrophils and macrophages can directly recognize invading pathogens through highly conserved structural constituents such as mannose and lipopolysaccharides (LPS) (Stahl and Ezekowitz, 1998; Ulevitch and Tobias, 1999). These structural motifs, referred to as pathogen-associated molecular patterns (PAMPs), are shared by large groups of pathogens because they perform essential functions in the microorganisms (Medzhitov and Janeway, 1997b). Recognition of PAMPs by specific receptors on phagocytes leads to direct engulfment of the microorganisms as well as activation of the phagocytic cells, resulting in the release of bactericidal proteins, peptides, and enzymes from pre-stored granules in neutrophils and release of chemokines and cytokines from monocytes and macrophages. These reactions together contribute to the development of an inflammatory response, which is characterized by vessel dilation and infiltration of additional phagocytes to the site of infection, as well as by systemic responses such as the synthesis of acute phase proteins. Examples of PAMP-engaging receptors on phagocytic cells include the mannose and scavenger receptors on macrophages (Dunne et al., 1994; Stahl and Ezekowitz, 1998) and the LPS receptor, CD14, on monocytes (Wright et al., 1990). Whereas phagocytic cells act to combat free-existing pathogens in the blood stream or tissues, natural killer cells fight viruses that have already taken residence inside the host cells by causing the destruction of cells that have been infected with the virus (Biron, 1997).

Complement is another major component of innate immunity. Although it is now taken for granted, the concept that complement functions as a major effector system of innate immunity has emerged only gradually. Indeed, complement-mediated cytolytic activity was initially described as an accessory reaction to antigen–antibody interactions (hence the term ''complement''). Later, it was found that in addition to the classical antibody-dependent pathway of activation, complement can also be activated by an ''alternative pathway'' that is triggered by susceptible foreign surfaces such as bacteria and yeast cell walls (Lambris, 1990; Lambris et al., 1998; Volanakis and Frank, 1998; Morgan and Harris, 1999). Just in the last decade, a third pathway, the lectin pathway, which probably is as ancient as the alternative pathway, was discovered (Matsushita and Fujita, 1995). All these findings suggest that complement functions as a constitutive mechanism at the front line of host defense. As we will discuss below, complement is also more than just a first line of defense. It has gained a considerable degree of sophistication during the course of evolution, enabling it to interact efficiently with other effector systems of innate immunity as well as those of adaptive immunity.

## 3. Direct activation pathways of complement by invading pathogens

Complement can be directly activated in response to an encounter with invading pathogens. This activation can be achieved through the alternative pathway or the lectin pathway. In either case, the presence on the microorganisms' surfaces of pathogen-specific sugar residues (e.g. mannose) or the absence of the galactose and sialic acid residues that normally decorate mammalian glycoproteins contribute to the tendency of microorganisms to trigger complement activation (Matsushita, 1996). In the case of the alternative pathway, the lack of sialic acid, for example on the bacterial cell wall, decreases the binding affinity of factor H, which is a cofactor for the C3b-degrading enzyme factor I. This situation, coupled with the absence of membrane complement regulating proteins (Hourcade et al., 1989), ensures that once C3b is spontaneously cross-linked onto the bacterial cell wall, it remains stable and serves as a

nidus for rapid amplification of the alternative pathway C3 convertase, C3bBb. In the lectin pathway, complement is activated by a serum protein, mannose-binding lectin (MBL), which is able to bind particular carbohydrates such as mannose or *N*-acetylglucosamine on the surfaces of microorganisms (Kawasaki et al., 1983; Turner, 1996). After binding to the sugars on the bacterial surface, MBL associates with and activates specific serine proteases, MBL-associated serine protease (MASP-1 and MASP-2) (Matsushita and Fujita, 1992; Thiel et al., 1997). Activated MASP will cleave C4 and C2, thereby activating the classical pathway of the complement cascade (Matsushita, 1996; Reid et al., 1998). Additionally, MASP1 was shown also to have the ability to cleave C3 and subsequently activates the alternative pathway (Matsushita and Fujita, 1995).

Not only can complement be activated directly by structural cues on the surfaces of microorganisms, but activated complement alone can also accomplish the task of eliminating invading pathogens through direct lysis. Generation of C3 convertase, either by the alternative pathway or the lectin pathway, will eventually lead to the assembly of the membrane attack complex (MAC) on the surfaces of the invading pathogens (Volanakis and Frank, 1998; Morgan and Harris, 1999; Lambris and Holers, 2000). MAC assembly then leads to membrane leakage and lysis of the bacteria or viral particles. Thus, complement can be self-sufficient as a form of innate immunity, in that it is able to defend the host against pathogen invasion without participation from any of the components of adaptive immunity.

## 4. Complement is present in lower as well as in higher animals

The nature of complement as a form of innate immunity is also demonstrated by its presence in lower animal species, which lack or have limited adaptive immunity. Although innate immune mechanisms such as fungicidal and bactericidal peptides and proteins, the prophenoloxidase activating system (ProPO), and lectins are present in primitive life forms (Ratcliffe et al., 1984; Cooper et al., 1992; Vasta et al., 1994; Magor and Vasta, 1998), mechanisms of adaptive immune defense such as immunoglobulins (Marchalonis et al., 1996), the major histocompatibility complex (Flajnik and Salter-Cid, 1995) and T-cell receptors (Rast and Litman, 1994) are first found in the cartilaginous fish (Warr, 1995; Litman and Rast, 1996). Recent studies, however, have identified several structural and functional counterparts of mammalian complement components in invertebrates. For example, C3-like and factor B (Al-Sharif et al., 1997; Smith et al., 1998) molecules have been detected in an echinoderm, the sea urchin. Also, a more evolved invertebrate, the tunicate, has been reported to possess C3 (Nonaka et al., 1999) and MASP (Matsushita et al., 1998). It seems reasonable to conclude, therefore, that primordial forms of the alternative and lectin pathways of complement activation had already taken shape over 700 million years ago.

Complement activity (alternative pathway) and complement components (C3 and factor B) have been characterized in the most primitive vertebrates, the jawless fish, although the classical pathway and the MAC have not yet been found in this species (Lambris et al., 1994; Matsushita et al., 1998; Sunyer and Lambris, 1998; Sunyer et al., 1998). The cartilaginous fish, and all vertebrates, including teleost fish, amphibians, reptiles, birds and mammals have developed all three pathways of complement activation (Fujii et al., 1992; Nonaka and Takahashi, 1992; Nonaka et al., 1994; Lambris et al., 1994). It is of great interest that C3 in teleost fish shows a high degree of complexity that is not commonly seen in mammalian species (Sunyer et al., 1998). For example, the rainbow trout, a quasi-tetraploid species, contains at least three different isoforms of C3 (Sunyer et al., 1996). Multiple C3 isoforms have also been characterized in the sea bream, a diploid fish (Sunyer et al., 1997a,b) and in the common carp where up to eight different forms of C3 have been described (Nakao et al., 2000). These various isoforms of C3 molecule have been shown to vary in their function, as indicated by the differences in their binding efficiencies to various complement-activating surfaces such as erythrocytes, *Escherichia coli*, and zymosan particles (Sunyer et al., 1996, 1997b). In addition to its increased number of C3 isoforms, complement in fish is also distinctive in being readily activated at low temperatures. Moreover, the alternative pathway complement activity is 5- to

10-fold higher in these creatures than in higher vertebrates (Sunyer and Tort, 1995). A diversified C3 repertoire, coupled with a higher titer and activity, may greatly expand the capacity of complement as a form of innate immunity to defend these animals against microorganisms in the aquatic environment. This mechanism confers a survival advantage upon these animals that have developed only limited adaptive immunity.

## 5. Interaction with other effectors of the innate immune system

Complement has co-evolved with other forms of innate immunity. Thus, it is not surprising to find close and synergistic interactions between complement and other effectors of the innate immune response. Indeed, MAC-mediated direct lysis of viruses and bacteria is only one of the three ways by which complement fulfils its role as a host defense mechanism. Activation of complement through any one of the three pathways leads to generation of the highly potent proinflammatory mediators C3a and C5a (Ember et al., 1998). These molecules are small peptide fragments cleaved from C3 and C5, respectively, and are commonly known as anaphylatoxins. Both C3a and C5a bind to specific G-protein coupled membrane receptors on phagocytes and cause their migration or activation (Wetsel, 1995; Ember et al., 1998). C5a is a well-characterized chemotactic agent that attracts neutrophils, eosinophils and other phagocytic cells to the sites of infection (Ember et al., 1998). Although C3a is not a chemotactic factor for neutrophils, it is capable of attracting eosinophils and mast cells, and it acts as an activator for mast cell degranulation (Daffern et al., 1995; Nilsson et al., 1996). The essential nature of the cooperation between complement and the cellular (phagocytic) mechanism of innate immunity was amply illustrated by the study of complement-deficient mice subjected to cecal-ligation and puncture (CLP) in an in vivo model of acute peritonitis. When compared to wild-type mice, the complement-deficient mice had a much lower survival rate because of their impaired ability to clear bacterial infection. This impairment was due at least in part to a reduced neutrophil infiltration into the peritoneum and impaired mast cell degranulation (Prodeus et al., 1997). A second way in which complement cooperates with phagocytic cells is through opsonization of invading pathogens. Covalent cross-linking of C3b fragments onto the bacterial surfaces not only initiates the cascade leading to MAC assembly, but also enables the C3b- or iC3b-decorated pathogens to be easily recognized by phagocytic cells bearing appropriate C3 receptors (CR1/CR3) (Ahearn and Fearon, 1989; Fallman et al., 1993; Xia and Ross, 2000).

Not only can complement activation synergize and promote other effector pathways of the innate immune response, but other innate immune mechanisms can also affect the complement system. For example, both MBL and C-reactive protein (CRP) are acute-phase proteins that are capable of interacting with the complement system and whose levels in the serum increase under inflammatory conditions (Claus et al., 1976; Thiel et al., 1992; Epstein et al., 1996; Szalai et al., 1997). As described above, MBL acts as a key protein for initiating the lectin pathway of complement activation. CRP can bind to a group of widely distributed ligands such as phosphocholine and to certain polycations and polysaccharides found on the surface of bacteria and of mammalian necrotic tissues (Szalai et al., 1997). It is well known that CRP activates complement via the classical pathway at the level of C1q (Gewurz et al., 1995; Szalai et al., 1997). CRP has also been shown to increase the binding of the complement regulatory protein H to C3b, thereby influencing the alternative pathway of complement activation (Suankratay et al., 1998). In another study, IL-6 was also found to dramatically upregulate C3 synthesis in vivo in the mouse (Kopf et al., 1998). Moreover, interferon (IFN)-gamma and -alpha, colony stimulating factor-1 (CSF-1), IL-6 and TNF-$\alpha$ have all been demonstrated to induce C1-INH synthesis in several cell types (Prada et al., 1998). IFN-$\alpha$ has also been implicated in the de novo synthesis of factor H by human mesangial cells, which also synthesize C3 upon stimulation by IL-1 (van den Dobbelsteen et al., 1994).

## 6. Interaction with adaptive immunity

A new model of vertebrate immunity has been hypothesized in which activation of the innate immunity provides an essential co-stimulatory signal for

the body to mount an effective acquired immune response (Bendelac and Fearon, 1997; Medzhitov and Janeway, 1997b). While from the viewpoint of complement biology the concept of interplay between the two forms of immunity is not new, since the classical pathway of complement activation is dependent on antibody–antigen engagement, recent studies using complement-deficient mice have highlighted the degree of interconnection between complement and several adaptive immune reactions (Carroll, 1998; Carroll and Prodeus, 1998). These studies have provided strong supporting evidence for the new paradigm outlined above concerning vertebrate immunity.

Natural antibodies, produced by $CD5^+$ B cells (or B-1 cells), are pre-existing antibodies that tend to bind with low affinity to many ligands, mainly polysaccharides but also endogenous neoantigens, and they assist the classical pathway of complement activation with the recognition of pathogens (Carroll, 1998; Carroll and Prodeus, 1998). The biological significance of natural antibodies has been revealed through the use of complement-deficient mice in a model of systemic infection. Both C4- and C3-deficient mice have impaired ability to clear LPS from the bloodstream (Fischer et al., 1997). This finding suggests that the classical pathway is normally involved in pathogen clearance. This conclusion has been further supported by the observation that mice deficient in IgM antibodies (RAG-2 nullizygous) also fail to clear LPS (Reid et al., 1997). In another model of bacterial infection, the CLP-induced acute septic peritonitis model, the classical pathway of complement (mediated by natural IgM antibodies) is required for recognition and efficient clearance of enteric bacteria (Prodeus et al., 1997). The presence of readily available pathogen-reactive natural antibodies in vertebrate animals would be expected to enhance the efficiency of complement activation as a first-line response to infection.

The interaction between complement and natural antibodies appears to be reciprocal; activation of complement is, in fact, also necessary for maintaining the titer and/or repertoire of natural antibodies (Ahearn et al., 1996). Thus, mice deficient in CD21/CD35 (Cr2 nullizygous, without the complement receptors CR1 and CR2) lack *E. coli*- and neoantigen-specific IgM natural antibodies and are as sensitive to CLP-induced acute septic peritonitis (Prodeus et al., 1997) or to ischemic/reperfusion injury of skeletal muscle (Weiser et al., 1996) as are C4- and C3-deficient mice. Indeed, the population of $CD5^+$ B-1 cells in the peritoneum was found to be significantly reduced in mice deficient in CD21/CD35 (Ahearn et al., 1996). These observations are consistent with the theory that complement opsonization of antigen and subsequent engagement of C3d–antigen complexes with the B-cell co-receptor are important for activation and expansion of B-1 cells (Carroll, 1998; Carroll and Prodeus, 1998).

The adjuvant role of complement in antibody production is not limited to the generation of natural antibodies. In a number of studies using C3-, C4- or CD21/35-deficient mice, decreased humoral responses to conventional thymus-dependent antigens were also observed (Fischer et al., 1996, 1998; Molina et al., 1996). This abnormality reflects impairment in follicular retention as well as germinal center survival during the process of B-cell activation within the secondary lymphoid compartment (Fischer et al., 1998). The impact of complement deficiencies appears to be specific to the function of B-lymphocytes, as the T-cell response remains normal in such animals (Fischer et al., 1996; Carroll and Prodeus, 1998). In the mouse, increased C3 synthesis after IL-6 stimulation has also been shown to contribute to the effect of IL-6 on germinal center development and antibody production in response to T cell-dependent antigen challenge (Kopf et al., 1998). These studies have collectively helped to build a widely accepted theory, which explains the mechanisms by which complement shapes the B-cell response. The iC3b/C3dg/C3d receptor CR2 (CD21) on B cells forms a complex with CD19 and CD81, known as the B-cell co-receptor. Activation of the complement system leads to covalent attachment of the C3 fragments iC3b, C3dg, and/or C3d to an antigen. This tagged antigen is then able to bind both the B-cell receptor (BCR) and co-receptor. Cross-linking of these receptors results in CD19 phosphorylation by BCR-associated kinases, triggering a series of intracellular signaling cascades that activate the B-lymphocyte (Tsokos et al., 1990; Tooze et al., 1997; Carroll, 1998; Carroll and Prodeus, 1998). It has been established using complement- and CD21-deficient mice that this co-liga-

tion between complement fragments and their receptor can lower the amount of antigen required for B-cell activation by 10- to 100-fold (Fischer et al., 1998). Thus, a co-stimulatory signal provided by complement tagging is considered to be essential for eliciting a humoral response to low-affinity antigens. Moreover, it has been demonstrated that complement participation is necessary for maintaining germinal center survival in the case of both low- and high-affinity antigens (Fischer et al., 1998). Additional evidence has been provided by a separate study involving a different approach, attachment of multiple copies of C3d to a model antigen, hen egg lysozyme. This attachment has been shown to lower the threshold of the immunogenic response in mice by two to three orders of magnitude (Dempsey et al., 1996). Finally, binding of the antigen–iC3b/C3dg/C3d complex to the CR2 receptor on antigen-presenting cells, e.g. mainly follicular dendritic cells (FDC) may enhance antigen presentation to B-lymphocytes (Carroll and Prodeus, 1998; Fang et al., 1998). This interaction between complement and antigen-presenting cells may facilitate the formation of germinal centers, modulate B-cell tolerance to self-antigens (Carroll and Prodeus, 1998; Noorchashm et al., 1999), and increase the persistence of B-cell memory (Carroll and Prodeus, 1998).

Complement-mediated clearance of immune complexes is another type of interaction between complement and adaptive immunity with serious pathophysiological implications (Atkinson, 1988; Morgan and Walport, 1991; Botto et al., 1998). As a result of immune recognition, antibodies attach to their target antigens in the circulation, forming immune complexes. Usually, these complexes bind to their Fc receptors and are eliminated from the circulation by Fc receptor-bearing effector cells. Excessive accumulation of immune complexes is a hallmark of autoimmune diseases. These complexes may eventually be deposited on the internal surfaces of blood vessels throughout the body, and particularly in the renal glomeruli, causing vasculitis and glomerulonephritis.

The complement system is known to play a critical role in the clearance of immune complexes (Atkinson, 1988; Morgan and Walport, 1991), which activate complement through the classical pathway. This activation leads to the covalent attachment of C3b and C4b to the complexes, thus facilitating their solubilization (Lambris, 1990; Volanakis and Frank, 1998; Morgan and Harris, 1999; Lambris and Holers, 2000). Moreover, cross-linked C3b and C4b can bind to the CR1 receptor on erythrocytes and phagocytes, directing the immune complexes to the reticuloendothelial system, where they are safely removed from circulation. Several factors are thought to affect the efficiency of clearance mediated by complement, such as the abundance of CR1 on the erythrocyte surface, and the type of antibody that forms the immune complex (Yokoyama and Waxman, 1992; Gibson and Waxman, 1994; Gonzalez and Waxman, 2000).

## 7. Self and non-self recognition and evasion strategies of microorganisms

Like other innate immune effectors, complement is capable of differentiating self from non-self using a number of mechanisms. In the lectin pathway, complement activation is initiated only after MBL binds its target molecules on the surfaces of microorganisms (Matsushita, 1996). The alternative pathway is spontaneously active and self-perpetuating. What makes this pathway proceed on the surfaces of microorganisms and not on those of host cells are the specific inhibitory constituents on the host cells. These include eukaryotic cell-specific sugar residues, such as sialic acid, and membrane-bound complement regulatory proteins, such as decay-accelerating factor (DAF), membrane cofactor protein (MCP), and CD59 (Hourcade et al., 1989). Activation of the classical pathway is dependent on antibodies, which, by themselves provide a high degree of specificity.

Although innate immunity is critical in providing the first line of defense, improper or exaggerated responses can cause great harm to the host. For example, production of inflammatory cytokines assists in the mobilization of host defense, but release of excessive amounts of such cytokines such as TNF-$\alpha$ into the systemic circulation can cause vessel dilation and plasma leakage, which may ultimately lead to fatal septic shock. As much appreciated as its beneficial function, the potential of activated complement to cause substantial autologous damage has also been a focus of investigation over the years (Lambris, 1990; Volanakis and Frank, 1998; Lambris and Holers, 2000). There are two typical conditions

under which complement-mediated self-destruction may occur. One is the autoimmune setting, which includes diseases such as lupus in which self-reacting antibodies are produced and deposited in vital tissue sites. This situation may trigger the activation of the classical pathway of complement and exacerbate Fc receptor-mediated inflammatory reaction. The second setting involves situations in which membrane complement regulating proteins are down-regulated, or are absent as a result of genetic mutation (Rosse and Parker, 1985; Yamashina et al., 1990). These challenges will render host cells susceptible to complement damage initiated either by the alternative pathway (spontaneous tick over or bystander activation resulting from infection) or by the classical pathway (through autoantibodies in autoimmune diseases or natural antibodies specific for neoantigens exposed during tissue ischemia/reperfusion). Studies of complement-deficient mice have greatly increased our understanding of complement as an important mechanism of innate immunity against foreign pathogens. The generation of new models in which mice lack critical membrane complement regulators, as reported recently (Sun et al., 1999; Xu et al., 2000), has now set the stage for defining the interaction between complement and the host tissues. Characterization of these and other yet-to-be-generated mutant mice, such as MCP- and CD59-deficient mice, may reveal novel mechanisms in the pathogenesis of inflammatory and autoimmune disorders.

It is interesting that while host cells have developed strategies to inhibit autologous complement activation, certain pathogens have also developed tricks that allow them to evade complement attack or to use complement components to facilitate their entry into host cells. These strategies include the avoidance of recognition or steric hindrance, removal of assembled complement complexes, and inhibition of complement activation. Avoidance of recognition is accomplished by the presence of non-activating molecules on the surface of encapsulated pathogens. These molecules include glycoproteins and lipophosphoglycans, or sialic acids (Joiner, 1988). Sialic acids favor the binding of factor H to the pathogen's surface, making activated C3b susceptible to cleavage by factor I. Furthermore, gram-positive bacterial peptidoglycans, or gram-negative bacterial outer membrane moieties (Merino et al., 1992) and capsules (Joiner, 1988) may hinder the access of complement proteins to the surface of the pathogen.

In some other cases, such as *E. coli* (Joiner, 1988) or *Leishmania major* (Puentes et al., 1990), the microorganisms have developed a strategy of shedding the assembled C5b-9 MAC from their surfaces. In yet another strategy, a pathogen may, through the process of natural selection, obtain proteins that inhibit the host complement. These proteins can be of host cell origin, or encoded in the pathogens' own genome. An example of the former is the acquisition of the human cellular proteins DAF and CD59 by HIV upon budding from the infected cell (Saifuddin et al., 1997; Stoiber et al., 1997). Similarly, Vaccinia Virus avails itself of host cells' DAF, CD59 and MCP (Vanderplasschen et al., 1998). Both of these strategies enable the pathogen to control complement activation on its surface. Other pathogens express their own complement regulatory proteins. For example, HSV-1 glycoprotein gC enables the virus to inhibit the binding of properdin to C3b, thus destabilizing the C3 convertase (McNearney et al., 1987; Kostavasil et al., 1997; Lubinski et al., 1999). Likewise, Vaccinia Virus (Sahu et al., 1998), Human Herpes Virus 8 (Russo et al., 1996), and Herpes Virus Saimiri (ORF-4) (Albrecht and Fleckenstein, 1995; Fodor et al., 1995) have developed a molecular mimicry strategy, by expressing structural homologues of mammalian complement control proteins. The best-characterized viral homologue thus far is VCP. This viral protein acts as a cofactor for factor I cleavage of C3. It therefore parallels the function of factor H and CR1, although it has been suggested that the interaction of VCP with C3 may be different from that of factor H and CR1 (Sahu et al., 1998).

Several invading pathogens use cellular complement receptors or regulatory proteins to initiate infection. This may be achieved by direct binding of a pathogenic protein to the cellular receptor or regulator. For example, the viral glycoprotein gp350 of Epstein-Barr Virus attaches to CR2 to infect B- and T-lymphocytes (Tsoukas and Lambris, 1993). Furthermore, gp350 bears sequence similarity to C3d and competes with this activation product of C3 for binding to its receptor. Examples of pathogens using membrane complement regulatory proteins include measles virus and *Neisseira gonorhoeae* (Cooper,

1991; Iwata et al., 1995), which use MCP as a receptor; and echoviruses (Bergelson et al., 1994), which use human DAF as a cellular receptor to mediate their entry.

## 8. Summary and concluding remarks

Innate immunity provides the first line of host defense. It also provides essential signals to ensure proper responses by the organism's adaptive immunity. Complement, one of the important effector systems of innate immunity, is present in lower as well as in higher animals. Some lower animal species such as teleost fish have a well-developed complement system with diversified complement proteins and enhanced efficiency that befits their particular living environment. Complement interacts critically with other mechanisms of innate immunity as well as with adaptive immunity to enhance host defense.

Finally, a few remarks about several new developments in the field that do not fall into the realm of classical complement functions: There is some evidence to suggest that complement and its membrane regulators can play important roles in reproductive biology. For example, both complement components C3 and DAF are synthesized in the mouse uterus in an estrogen-regulated manner (Sundstrom et al., 1989; Sun et al., 1999). Expression of DAF, Crry, MCP and CD59 has also been demonstrated in several other mammalian reproductive tissues, such as the trophoblast and the testis (Hasty et al., 1991; Cervoni et al., 1993; Simpson and Holmes, 1994). Furthermore, a recent study on Crry knockout mice has revealed a critical role of this protein in fetal-maternal tolerance (Xu et al., 2000). In addition, participation of several complement components in tissue regeneration has recently been proposed. Expression of C3 was detected in regenerating limb blastema cells in two urodele species, where it was apparently associated with the process of dedifferentiation and redifferentiation of myoblast cells (Del Rio-Tsonis et al., 1998). In other studies, C5 has been implicated in liver tissue regeneration after toxic exposure to $CCl_4$ (Mastellos et al., 2000) and in neuronal protection against degenerative stimuli (e.g. excitotoxin KA Kaininc Acid) in the murine CNS (Pasinetti et al., 1996). With the renewed interest in complement research that we have recently witnessed, rapid and exciting advances in these areas, as well as in the more traditional roles of complement in host defense, can be expected in the future.

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