

Review

Complement and innate immunity

Wen-Chao Song^{a,1}, Maria Rosa Sarrias^b, John D. Lambris^{b,*}

^a Center for Experimental Therapeutics and Department of Pharmacology, University of Pennsylvania School of Medicine, 1351 BRBII / III, 421 Curie Blvd., Philadelphia, PA 19104, USA

^b Protein Chemistry Laboratory, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, 401 Stellar Chance Laboratories, Philadelphia, PA 19104, USA

Accepted 12 April 2000

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 In higher animals, innate immunity was once viewed as an evolutionary relic whose functions had largely been superseded by the advent of adaptive immunity.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

* Corresponding author. Tel.: +1-215-746-5766; fax: +1-215-573-8738.

E-mail address: lambris@mail.med.upenn.edu (J.D. Lambris).

¹ Tel.: +1-215-573-6641; fax: +1-215-573-5408.

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 immuno-

globulins (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)- γ and - α , colony stimulating factor-1 (CSF-1), IL-6 and TNF- α have all been demonstrated to induce C1-INH synthesis in several cell types (Prada et al., 1998). IFN- α 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- α 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 cap-

sules (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 (Vanderplassen 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 *Neisseria gonorrhoeae* (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 CCl₄ (Mastellos et al., 2000) and in neuronal protection against degenerative stimuli (e.g. excitotoxin KA Kainic 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.

Acknowledgements

This work was supported by National Institutes of Health grant GM 56698, and Cancer and Diabetes Centers Core Support Grants CA 16520 and DK 19525.

References

- Ahearn, J.M., Fearon, D.T., 1989. Structure and function of the complement receptors, CR1 (CD35), and CR2 (CD21). *Adv. Immunol.* 46, 183–219.
- Ahearn, J.M., Fischer, M.B., Croix, D., Goerg, S., Ma, M., Xia, J., Zhou, X., Howard, R.G., Rothstein, T.L., Carroll, M.C., 1996. Disruption of the Cr2 locus results in a reduction in B-1a cells and in an impaired B cell response to T-dependent antigen. *Immunity* 4, 251–262.
- Albrecht, J.C., Fleckenstein, B., 1995. In: McFadden, G. (Ed.), *Complement Regulatory Proteins of Herpesvirus Saimiri*. R.G. Landes, Austin, pp. 127–145.
- Al-Sharif, W.Z., Sunyer, J.O., Lambris, J.D., Smith, L.C., 1997. Sea urchin coelomocytes specifically express a homologue of the complement component C3. *J. Immunol.* 160, 2983–2997.
- Atkinson, J.P., 1988. Complement deficiency. Predisposing factor to autoimmune syndromes. *Am. J. Med.* 85, 45–47.
- Bendelac, A., Fearon, D.T., 1997. Innate pathways that control acquired immunity. *Curr. Opin. Immunol.* 9, 1–3.
- Bergelson, J.M., Chan, M., Solomon, K.R., Stjohn, N.F., Lin, H.M., Finberg, R.W., 1994. Decay-accelerating factor (CD55), a glycosylphosphatidylinositol-anchored complement regulatory protein, is a receptor for several echoviruses. *Proc. Natl. Acad. Sci. U.S.A.* 91, 6245–6249.
- Biron, C.A., 1997. Activation and function of natural killer cell responses during viral infections. *Curr. Opin. Immunol.* 9, 24–34.
- Borregaard, N., Elsbach, P., Ganz, T., Garred, P., Svejgaard, A., 2000. Innate immunity: from plants to humans. *Immunol. Today* 21, 68–70.
- Botto, M., Dell'Agnola, C., Bygrave, A.E., Thompson, E.M., Cook, H.T., Petry, F., Loos, M., Pandolfi, P.P., Walport, M.J., 1998. Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nat. Genet.* 19, 56–59.
- Carroll, M.C., 1998. The role of complement and complement

- receptors in induction and regulation of immunity. *Annu. Rev. Immunol.* 16, 545–568.
- Carroll, M.C., Janeway, C.A., 1999. Innate immunity. *Curr. Opin. Immunol.* 11, 11–12.
- Carroll, M.C., Prodeus, A.P., 1998. Linkages of innate and adaptive immunity. *Curr. Opin. Immunol.* 10, 36–40.
- Cervoni, F., Oglesby, T.J., Fenichel, P., Dohr, G., Rossi, B., Atkinson, J.P., Hsi, B.L., 1993. Expression of decay-accelerating factor (CD55) of the complement system on human spermatozoa. *J. Immunol.* 151, 939–948.
- Claus, D.R., Osmand, A.P., Gewurz, H., 1976. Radioimmunoassay of human C-reactive protein and levels in normal sera. *J. Lab. Clin. Med.* 87, 120–128.
- Cooper, N.R., 1991. Complement evasion strategies of microorganisms. *Immunol. Today* 12, 327–331.
- Cooper, E.L., Rinkevich, B., Uhlenbruck, G., Valembos, P., 1992. Invertebrate immunity — another viewpoint — editorial. *Scand. J. Immunol.* 35, 247–266.
- Daffern, P.J., Pfeifer, P.H., Ember, J.A., Hugli, T.E., 1995. C3a is a chemotaxin for human eosinophils but not for neutrophils: I. C3a stimulation of neutrophils is secondary to eosinophil activation. *J. Exp. Med.* 181, 2119–2127.
- Del Rio-Tsonis, K., Tsonis, P.A., Zarkadis, I.K., Tsangas, A.G., Lambris, J.D., 1998. Expression of the third component of complement, C3, in regenerating limb blastema cells of urodeles. *J. Immunol.* 161, 6819–6824.
- Dempsey, P.W., Allison, M.E.D., Akkaraju, S., Goodnow, C.C., Fearon, D.T., 1996. C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. *Science* 271, 348–350.
- Dunne, D.W., Resnick, D., Greenberg, J., Krieger, M., Joiner, K.A., 1994. The type I macrophage scavenger receptor binds to gram-positive bacteria and recognizes lipoteichoic acid. *Proc. Natl. Acad. USA* 91, 1863–1867.
- Ember, J.A., Jagels, M.A., Hugli, T.E., 1998. In: Volanakis, J.E., Frank, M.M. (Eds.), *Characterization of Complement Anaphylatoxins and their Biological Responses*. Marcel Dekker, New York, pp. 241–284.
- Epstein, J., Eichbaum, Q., Sheriff, S., Ezekowitz, R.A., 1996. The collectins in innate immunity. *Curr. Opin. Immunol.* 8, 29–35.
- Fallman, M., Andersson, R., Andersson, T., 1993. Signaling properties of CR3 (CD11b/CD18) and CR1 (CD35) in relation to phagocytosis of complement-opsonized particles. *J. Immunol.* 151, 330–338.
- Fang, Y.F., Xu, C.G., Fu, Y.X., Holers, V.M., Molina, H., 1998. Expression of complement receptors 1 and 2 on follicular dendritic cells is necessary for the generation of a strong antigen-specific IgG response. *J. Immunol.* 160, 5273–5279, June 1.
- Fearon, D.T., 1997. Seeking wisdom in innate immunity. *Nature* 388, 323–324.
- Fearon, D.T., Locksley, R.M., 1996. The instructive role of innate immunity in the acquired immune response. *Science* 272, 50–53.
- Fischer, M.B., Ma, M., Goerg, S., Zhou, X., Xia, J., Finco, O., Han, S., Kelsoe, G., Howard, R.G., Rothstein, T.L., Kremmer, E., Rosen, F.S., Carroll, M.C., 1996. Regulation of the B cell response to T-dependent antigens by classical pathway complement. *J. Immunol.* 157, 549–556.
- Fischer, M.B., Prodeus, A.P., Nicholson-Weller, A., Ma, M.H., Murrow, J., Reid, R.R., Warren, H.B., Lage, A.L., Moore, F.D.J., Rosen, F.S., Carroll, M.C., 1997. Increased susceptibility to endotoxin shock in complement C3- and C4-deficient mice is corrected by C1 inhibitor replacement. *J. Immunol.* 159, 976–982.
- Fischer, M.B., Goerg, S., Shen, L., Prodeus, A.P., Goodnow, C.C., Kelsoe, G., Carroll, M.C., 1998. Dependence of germinal center B cells on expression of CD21/CD35 for survival. *Science* 280, 582–585.
- Flajnik, M.F., Salter-Cid, L., 1995. Evolution and developmental regulation of the major histocompatibility complex. *Crit. Rev. Immunol.* 15, 31–75.
- Fodor, W.L., Rollins, S.A., Biancocarson, S., Rother, R.P., Guilmette, E.R., Burton, W.V., Albrecht, J.C., Fleckenstein, B., Squinto, S.P., 1995. The complement control protein homolog of herpesvirus saimiri regulates serum complement by inhibiting C3 convertase activity. *J. Virol.* 69, 3889–3892.
- Fujii, T., Nakamura, T., Sekizawa, A., Tomonaga, S., 1992. Isolation and characterization of a protein from hagfish serum that is homologous to the 3rd component of the mammalian complement system. *J. Immunol.* 148, 117–123.
- Gewurz, H., Zhang, X.H., Lint, T.F., 1995. Structure and function of the pentraxins. *Curr. Opin. Immunol.* 7, 54–64.
- Gibson, N.C., Waxman, F.J., 1994. Relationship between immune complex binding and release and the quantitative expression of the complement receptor, type 1 (Cr1, Cd35) on human erythrocytes. *Clin. Immunol. Immunopathol.* 70, 104–113.
- Gonzalez, M.L., Waxman, F.J., 2000. Glomerular deposition of immune complexes made with IgG2a monoclonal antibodies. *J. Immunol.* 164, 1071–1077.
- Hasty, L.A., Lessey, B.A., Pruksananonda, S., Lambris, J.D., Lyttle, C.R., 1991. The hormonal regulation of complement components throughout the menstrual cycle. In: *Annual Meeting of the Society for Gynecologic Investigation*.
- Hoffmann, J.A., Kafatos, F.C., Janeway, C.A., Ezekowitz, R.A., 1999. Phylogenetic perspectives in innate immunity. *Science* 284, 1313–1318.
- Hourcade, D., Holers, V.M., Atkinson, J.P., 1989. The regulators of complement activation (RCA) gene cluster. *Adv. Immunol.* 45, 381–416.
- Iwata, K., Seya, T., Yanagi, Y., Pesando, J.M., Johnson, P.M., Okabe, M., Ueda, S., Ariga, H., Nagasawa, S., 1995. Diversity of sites for measles virus binding and for inactivation of complement C3b and C4b on membrane cofactor protein CD46. *J. Biol. Chem.* 270, 15148–15152.
- Joiner, K.A., 1988. Complement evasion by bacteria and parasites. *Annu. Rev. Microbiol.* 42, 201–230.
- Kawasaki, N., Kawasaki, T., Yamashina, I., 1983. Isolation and characterization of a mannan-binding protein from human serum. *J. Biochem. (Tokyo)* 94, 937–947.
- Kopf, M., Herren, S., Wiles, M.V., Pepys, M.B., Kosco-Vilbois, M.H., 1998. Interleukin 6 influences germinal center development and antibody production via a contribution of C3 complement component. *J. Exp. Med.* 188, 1895–1906.

- Kostavasil, I., Sahu, A., Friedman, H.M., Eisenberg, R.J., Cohen, G.H., Lambris, J.D., 1997. Mechanism of complement inactivation by glycoprotein C of herpes simplex virus. *J. Immunol.* 158, 1763–1771.
- Lambris, J.D., 1990. *The Third Component of Complement: Chemistry and Biology*. Springer, Berlin.
- Lambris, J.D., Holers, V.M., 2000. *Therapeutic Interventions in the Complement System*. Humana Press, Totawa, NJ, USA.
- Lambris, J.D., Mavroidis, M., Sunyer, J.O., 1994. In: Erdei, A. (Ed.), *Phylogeny of Third Component of Complement*, C3. R.G. Landes, Austin, pp. 15–34.
- Lambris, J.D., Sahu, A., Wetsel, R., 1998. In: Volanakis, J.E., Frank, M. (Eds.), *The Chemistry and Biology of C3, C4, and C5*. Marcel Dekker, New York, pp. 83–118.
- Litman, G.W., Rast, J.P., 1996. The organization and structure of immunoglobulin and T-cell receptor genes in the most phylogenetically distant jawed vertebrates: evolutionary implications. *Res. Immunol.* 147, 226–233.
- Lubinski, J., Wang, L., Mastellos, D., Sahu, A., Lambris, J.D., Friedman, H.M., 1999. In vivo role of complement-interacting domains of herpes simplex virus type 1 glycoprotein gC. *J. Exp. Med.* 190, 1637–1646.
- Magor, K.E., Vasta, G.R., 1998. Ancestral immunity comes of age. *Immunol. Today* 19, 54–56.
- Marchalonis, J.J., Bernstein, R.M., Shen, S.X., Schluter, S.F., 1996. Emergence of the immunoglobulin family. Conservation in protein sequence and plasticity in gene organization. *Glycobiology* 6, 657–663.
- Mastellos, D., Papageorgiou, J., Franchini, S., Tsonis, P.A., Lambris, J.D., 2000. A role of complement component C5 in liver regeneration. *Immunopharmacology*, in press.
- Matsushita, M., 1996. The lectin pathway of the complement system. *Microbiol. Immunol.* 40, 887–893.
- Matsushita, M., Fujita, T., 1992. Activation of the classical complement pathway by mannose-binding protein in association with a novel C1s-like serine protease. *J. Exp. Med.* 176, 1497–1502.
- Matsushita, M., Fujita, T., 1995. Cleavage of the third component of complement (C3) by mannose-binding protein-associated serine protease (MASP) with subsequent complement activation. *Immunobiology* 194, 443–448.
- Matsushita, M., Endo, Y., Nonaka, M., Fujita, T., 1998. Complement-related serine proteases in tunicates and vertebrates. *Curr. Opin. Immunol.* 10, 29–35.
- McNearney, T.A., Odell, C., Holers, V.M., Spear, P.G., Atkinson, J.P., 1987. Herpes simplex virus glycoproteins gC-1 and gC-2 bind to the third component of complement and provide protection against complement-mediated neutralization of viral infectivity. *J. Exp. Med.* 166, 1525–1535.
- Medzhitov, R., Janeway, C.A. Jr., 1997a. Innate immunity: impact on the adaptive immune response. *Curr. Opin. Immunol.* 9, 4–9.
- Medzhitov, R., Janeway, C.A. Jr., 1997b. Innate immunity: the virtues of a nonclonal system of recognition. *Cell* 91, 295–298.
- Merino, S., Campubri, S., Alberti, S., Benedi, V.J., Tomas, J.M., 1992. Mechanisms of *Klebsiella pneumoniae* resistance to complement-mediated killing. *Infect. Immun.* 60, 2529–2535.
- Molina, H., Holers, V.M., Li, B., Fung, Y., Mariathasan, S., Goellner, J.S.-S.J., Karr, R.W., Chaplin, D.D., 1996. Markedly impaired humoral immune response in mice deficient in complement receptors 1 and 2. *Proc. Natl. Acad. Sci. U.S.A.* 93, 3357–3361.
- Morgan, B.P., Harris, C.L., 1999. *Complement Regulatory Proteins*. Academic Press.
- Morgan, B.P., Walport, M.J., 1991. Complement deficiency and disease. *Immunol. Today* 12, 301–306.
- Nakao, M., Mutsuro, J., Obo, R., Fujiki, K., Nonaka, M., Yano, T., 2000. Molecular cloning and protein analysis of divergent forms of the complement component C3 from a bony fish, the common carp (*Cyprinus carpio*): presence of variants lacking the catalytic histidine. *Eur. J. Immunol.* 30, 858–866.
- Nilsson, G., Johnell, M., Hammer, C.H., Tiffany, H.L., Nilsson, K., Metcalfe, D.D., Siegbahn, A., Murphy, P.M., 1996. C3a and C5a are chemotaxins for human mast cells and act through distinct receptors via a pertussis toxin-sensitive signal transduction pathway. *J. Immunol.* 157, 1693–1698.
- Nonaka, M., Takahashi, M., 1992. Complete complementary DNA sequence of the 3rd component of complement of Lamprey: implication for the evolution of thioester containing proteins. *J. Immunol.* 148, 3290–3295.
- Nonaka, M., Takahashi, M., Sasaki, M., 1994. Molecular cloning of a Lamprey homologue of the mammalian MHC class III gene, complement factor B. *J. Immunol.* 152, 2263–2269.
- Nonaka, M., Azumi, K., Ji, X., Namikawa-Yamada, C., Sasaki, M., Saiga, H., Dodds, A.W., Sekine, H., Homma, M.K., Matsushita, M., Endo, Y., Fujita, T., 1999. Opsonic complement component C3 in the solitary ascidian, *Halocynthia roretzi*. *J. Immunol.* 162, 387–391.
- Noorchashm, H., Moore, D.J., Lieu, Y.K., Noorchashm, N., Schlachterman, A., Song, H.K., Lambris, J.D., Barker, C.F., Naji, A., 1999. Contribution of the innate immune system to autoimmune diabetes: a role for the CR1/CR2 complement receptors. *Cell. Immunol.* 195 (1), 75–79.
- Pasinetti, G.M., Tocco, G., Sakhi, S., Musleh, W.D., DeSimoni, M.G., Mascarucci, P., Schreiber, S., Baudry, M., Finch, C.E., 1996. Hereditary deficiencies in complement C5 are associated with intensified neurodegenerative responses that implicate new roles for the C-system in neuronal and astrocytic functions. *Neurobiol. Dis.* 3, 197–204.
- Prada, A.E., Zahedi, K., Davis, A.E. III, 1998. Regulation of C1 inhibitor synthesis. *Immunobiology* 199, 377–388.
- Prodeus, A.P., Zhou, X., Maurer, M., Galli, S.J., Carroll, M.C., 1997. Impaired mast cell-dependent natural immunity in complement C3-deficient mice. *Nature* 390, 172–175.
- Puentes, S.M., Dasilva, R.P., Sacks, D.L., Hammer, C.H., Joiner, K.A., 1990. Serum resistance of metacyclic stage leishmania-major promastigotes is due to release of C5b-9. *J. Immunol.* 145, 4311–4316.
- Rast, J.P., Litman, G.W., 1994. T-cell receptor gene homologs are present in the most primitive jawed vertebrates. *Proc. Natl. Acad. Sci. U.S.A.* 91, 9248–9252.
- Ratcliffe, N.A., Leonard, C., Rowley, A.F., 1984. Prophenoloxidase activation, non-self recognition and cell co-operation in insect immunity. *Science* 226, 557–559.

- Reid, R.R., Prodeus, A.P., Khan, W., Hsu, T., Rosen, F.S., Carroll, M.C., 1997. Endotoxin shock in antibody-deficient mice — unraveling the role of natural antibody and complement in the clearance of lipopolysaccharide. *J. Immunol.* 159, 970–975, July 15.
- Reid, K.B., Colomb, M.G., Loos, M., 1998. Complement component C1 and the collectins: parallels between routes of acquired and innate immunity. *Immunol. Today* 19, 56–59.
- Rosse, W.F., Parker, C.J., 1985. Paroxysmal nocturnal haemoglobinuria. *Clin. Haematol.* 14, 105–125.
- Russo, J.J., Bohenzky, R.A., Chien, M.C., Chen, J., Yan, M., Maddalena, D., Parry, J.P., Peruzzi, D., Edelman, I.S., Chang, Y., Moore, P.S., 1996. Nucleotide sequence of the Kaposi sarcoma-associated herpesvirus (HHV8). *Proc. Natl. Acad. Sci. USA* 93, 14862–14867.
- Sahu, A., Isaacs, S.N., Soulika, A.M., Lambris, J.D., 1998. Interaction of vaccinia virus complement control protein with human complement proteins: factor I-mediated degradation of C3b to iC3b(1) inactivates the alternative complement pathway. *J. Immunol.* 160, 5596–5604.
- Saifuddin, M., Hedayati, T., Atkinson, J.P., Holguin, M.H., Parker, C.J., Spear, G.T., 1997. Human immunodeficiency virus type 1 incorporates both glycosyl phosphatidylinositol-anchored CD55 and CD59 and integral membrane CD46 at levels that protect from complement-mediated destruction. *J. Gen. Virol.* 78, 1907–1911, (Part 8).
- Simpson, K.L., Holmes, C.H., 1994. Differential expression of complement regulatory proteins decay-accelerating factor (CD55), membrane cofactor protein (CD46) and CD59 during human spermatogenesis. *Immunology* 81, 452–461.
- Smith, L.C., Shih, C.S., Dachenhausen, S.G., 1998. Coelomocytes express SpBf, a homologue of factor B, the second component in the sea urchin complement system. *J. Immunol.* 161, 6784–6793.
- Stahl, P.D., Ezekowitz, R.A., 1998. The mannose receptor is a pattern recognition receptor involved in host defense. *Curr. Opin. Immunol.* 10, 50–55.
- Stoiber, H., Clivio, A., Dierich, M.P., 1997. Role of complement in HIV infection. *Annu. Rev. Immunol.* 15, 649–674.
- Suankratay, C., Mold, C., Zhang, Y., Potempa, L.A., Lint, T.F., Gewurz, H., 1998. Complement regulation in innate immunity and the acute-phase response: inhibition of mannan-binding lectin-initiated complement cytotoxicity by C-reactive protein (CRP). *Clin. Exp. Immunol.* 113, 353–359.
- Sun, X., Funk, C.D., Deng, C., Sahu, A., Lambris, J.D., Song, W.C., 1999. Role of decay-accelerating factor in regulating complement activation on the erythrocyte surface as revealed by gene targeting. *Proc. Natl. Acad. Sci. U.S.A.* 96, 628–633.
- Sundstrom, S.A., Komm, B.S., Ponce-de-Leon, H., Yi, Z., Teuscher, C., Lyttle, R.C., 1989. Estrogen regulation of tissue-specific expression of complement C3. *J. Biol. Chem.* 264 (28), 16941–16947.
- Sunyer, J.O., Lambris, J.D., 1998. Evolution and diversity of the complement system of poikilothermic vertebrates. *Immunol. Rev.* 166, 39–57.
- Sunyer, J.O., Tort, L., 1995. Natural haemolytic and bactericidal activities of sea bream (*Sparus aurata*) are effected by the alternative complement pathway. *Vet. Immunol. Immunopathol.* 45, 333–345.
- Sunyer, J.O., Zarkadis, I.K., Sahu, A., Lambris, J.D., 1996. Multiple forms of complement C3 in trout, that differ in binding to complement activators. *Proc. Natl. Acad. Sci. U.S.A.* 93, 8546–8551.
- Sunyer, J.O., Tort, L., Lambris, J.D., 1997a. Structural C3 diversity in fish — characterization of five forms of C3 in the diploid fish *Sparus aurata*. *J. Immunol.* 158, 2813–2821.
- Sunyer, J.O., Tort, L., Lambris, J.D., 1997b. Diversity of the third form of complement, C3, in fish: functional characterization of five forms of C3 in the diploid fish *Sparus aurata*. *Biochem. J.* 326, 877–881.
- Sunyer, J.O., Zarkadis, I.K., Lambris, J.D., 1998. Complement diversity: a mechanism for generating immune diversity?. *Immunol. Today* 19, 519–523.
- Szalai, A.J., Agrawal, A., Greenhough, T.J., Volanakis, J.E., 1997. C-reactive protein: structural biology, gene expression, and host defense function. *Immunol. Res.* 16, 127–136.
- Thiel, S., Holmskov, U., Hviid, L., Laursen, S.B., Jensenius, J.C., 1992. The concentration of the C-type lectin, mannan-binding protein, in human plasma increases during an acute phase response. *Clin. Exp. Immunol.* 90, 31–35.
- Thiel, S., Vorup-Jensen, T., Stover, C.M., Schwaebler, W., Laursen, S.B., Poulsen, K., Willis, A.C., Eggleton, P., Hansen, S., Holmskov, U., Reid, K.B., Jensenius, J.C., 1997. A second serine protease associated with mannan-binding lectin that activates complement. *Nature* 386, 506–510, April 3.
- Tooze, R.M., Doody, G.M., Fearon, D.T., 1997. Counterregulation by the coreceptors CD19 and CD22 of MAP kinase activation by membrane immunoglobulin. *Immunity* 7, 59–67.
- Tsokos, G.C., Lambris, J.D., Finkelman, F.D., Anastassiou, E.D., June, C.H., 1990. Monovalent ligands of complement receptor 2 inhibit whereas polyvalent ligands enhance anti-Ig-induced human B cell intracytoplasmic free calcium concentration. *J. Immunol.* 144, 1640–1645.
- Tsoukas, C.D., Lambris, J.D., 1993. Expression of EBV/C3d receptors on T cells: biological significance. *Immunol. Today* 14, 56–59.
- Turner, M.W., 1996. Mannose-binding lectin: the pluripotent molecule of the innate immune system. *Immunol. Today* 17, 532–540.
- Ulevitch, R.J., Tobias, P.S., 1999. Recognition of gram-negative bacteria and endotoxin by the innate immune system. *Curr. Opin. Immunol.* 11, 19–22.
- van den Dobbelaars, M.E., Verhasselt, V., Kaashoek, J.G., Timmerman, J.J., Schroeijsers, W.E., Verweij, C.L., van der Woude, F.J., van Es, L.A., Daha, M.R., 1994. Regulation of C3 and factor H synthesis of human glomerular mesangial cells by IL-1 and interferon-gamma. *Clin. Exp. Immunol.* 95, 173–180.
- Vanderplasschen, A., Mathew, E., Hollinshead, M., Sim, R.B., Smith, G.L., 1998. Extracellular enveloped vaccinia virus is resistant to complement because of incorporation of host complement control proteins into its envelope. *Proc. Natl. Acad. Sci. U.S.A.* 95, 7544–7549.
- Vasta, G.R., Ahmed, A., Fink, N.E., Elola, M.T., Marsh, A.G., Snowden, A., Odom, E.W., 1994. Animal lectins as

- self/non-self recognition molecules-biochemical and genetic approaches to understanding their biological roles and evolution. *Ann. N. Y. Acad. Sci.*, 55–73, New York.
- Volanakis, J.E., Frank, M., 1998. *The Human Complement System in Health and Disease*. Marcel Dekker, New York, NY.
- Warr, G.W., 1995. The immunoglobulin genes of fish. *Dev. Comp. Immunol.* 19, 1–12.
- Weiser, M.R., Williams, J.P., Moore, F.D., Kobzik, L., Ma, M.H., Hechtman, H.B., Carroll, M.C., 1996. Reperfusion injury of ischemic skeletal muscle is mediated by natural antibody and complement. *J. Exp. Med.* 183, 2343–2348.
- Wetsel, R.A., 1995. Structure, function and cellular expression of complement anaphylatoxin receptors. *Curr. Opin. Immunol.* 7, 48–53.
- Wright, S.D., Ramos, R.A., Tobias, P.S., Ulevitch, R.J., Mathison, J.C., 1990. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science* 249, 1431–1433, [see comments].
- Xia, Y., Ross, G.D., 2000. Roles of integrins CR3 and CR4 in disease and therapeutic strategies. In: Lambris, J.D., Holers, V.M. (Eds.), *Therapeutic Interventions in the Complement System*. Humana Press, Totawa, NJ, USA.
- Xu, C., Mao, D., Holers, V.M., Palanca, B., Cheng, A.M., Molina, H., 2000. A critical role for murine complement regulator *crry* in fetomaternal tolerance. *Science* 287, 498–501.
- Yamashina, M., Ueda, E., Kinoshita, T., Takami, T., Ojima, A., Ono, H., Tanaka, H., Kondo, N., Orii, T., Okada, N., 1990. Inherited complete deficiency of 20-kilodalton homologous restriction factor (CD59) as a cause of paroxysmal nocturnal hemoglobinuria. *N. Engl. J. Med.* 323, 1184–1189.
- Yokoyama, I., Waxman, F., 1992. Isotypic and clonal variations in the interactions between model monoclonal immune complexes and the human erythrocyte CR1-receptor. *Mol. Immunol.* 29, 935–947.