

A Regulatory Role for Complement in Innate Immunity and Autoimmunity

Alma J. Nauta Anja Roos Mohamed R. Daha

Leiden University Medical Center, Nephrology Department, Leiden, The Netherlands

Key Words

Complement · Apoptosis · Autoimmunity · Receptors · Dendritic cells

Abstract

The complement system comprises a strong defense against various pathogens and is a major component of our innate immune system. While earlier studies have established a crucial role of complement in recognition, opsonization and enhanced phagocytosis of microorganisms by professional phagocytes such as polymorphonuclear leukocytes and macrophages, recent studies delineate an additional role of complement in initiation and maintenance of the acquired immune response. In addition, it seems that opsonization of apoptotic cells by complement may lead to polarization of the response of professional antigen-presenting cells to a more inflammatory or tolerogenic response. The present review summarizes these different contributions of complement to the shaping of the immune balance.

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Molecules of the Innate Immune System

The immune system has evolved to protect the human body against pathogens or other hazardous elements including bacteria, viruses, parasites and tumors. Immune responses are mediated by a variety of cells and soluble

mediators and can be distinguished into innate and adaptive immune responses, the latter being highly specific for particular antigens. The adaptive immune responses can be divided in two broad classes: the humoral and the cell-mediated immune responses. In the humoral immune response, antibodies are produced by B lymphocytes, which specifically recognize and bind to a particular target molecule. The different responses of T lymphocytes are collectively called the cell-mediated immune responses. The innate immune system consists of physical, cellular and chemical elements. The physical elements include the epithelial and mucosal barriers, whereas natural killer (NK) cells, mast cells, dendritic cells, macrophages and granulocytes constitute the cellular elements of the innate immune system. The chemical defense consists of mucus, acid, cytokines, chemokines, proteins or peptides that hydrolyze microbes, and so-called pattern recognition molecules. The pattern recognition molecules have the capability to specifically recognize pathogens via their highly conserved pathogen-associated molecular patterns displayed by lipopolysaccharides, lipoproteins, peptidoglycans and oligosaccharides [1]. Originally, pattern recognition was thought to be primarily important in host defense by the recognition of exogenous ligands. However, recent data extend the role of these pattern recognition molecules to the recognition of endogenous ligands, indicating that they also play a role in the clearance of self-antigens [2]. Pattern recognition molecules can be cell-associated such as scavenger receptors, Toll-like receptors and lectin-like

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1018–2438/04/1344–0310\$21.00/0

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Correspondence to: Alma J. Nauta
Leiden University Medical Center
Nephrology Department, Albinusdreef 2
NL–2333 ZA Leiden (The Netherlands)
Tel. +31 71 526 2274, Fax +31 71 526 8118, E-Mail m.r.daha@lumc.nl

receptors. There are also several soluble molecules that bind pathogen-associated molecular patterns including complement components, members of the collectin family and members of the pentraxin family. The complement system is a key component of the innate immune system involved in protection against invading pathogens.

The Complement System

The complement system consists of at least 30 plasma proteins with an associated group of cell membrane proteins that have inflammatory and immunoregulatory functions. Many of the proteins are in a proenzymatic form and are only activated via proteolytic cleavage by complement components earlier in the cascade. The complement system consists of at least three different pathways that are initiated via separate mechanisms: the classical complement pathway, the alternative complement pathway, and the lectin complement pathway (fig. 1).

The three pathways merge at the level of C3, resulting in the generation of the membrane attack complex (MAC) through a common terminal pathway. The classical complement pathway is activated by binding of C1q to antibody-antigen complexes, or other structures, such as HIV [3], bacterial structures [4], double-stranded DNA [4, 5], or pentraxins such as C-reactive protein (CRP) [6]. Recognition of microbial saccharides by mannose-binding lectin (MBL) or ficolins can initiate activation of the lectin pathway [7]. The alternative pathway of complement is activated following the spontaneous hydrolysis of the thioester bond of native C3 and requires stabilization of activated complement components, for example, by bacteria or viruses [8]. The alternative pathway also provides an intrinsic amplification mechanism at the level of C3. The common terminal complement pathway results in the formation of the MAC, consisting of complement components C5–C9.

Because of the amplifying capacity of complement and the production of various inflammatory mediators, control mechanisms exist to prevent ongoing activation that could result in significant damage to host tissue. Different complement regulators, both fluid phase and membrane-bound, are present to tightly regulate the complement system [9]. However, undesired complement activation might occur and has been implicated in the pathogenesis of several diseases, including glomerulonephritis [10], myocardial infarction [11], and rheumatoid arthritis [12].

Being a central component of the innate immune system, complement is involved in host defense against invading pathogens, i.e. non-self. The classical view on the action of complement in the protection against pathogens is that it kills targets by the formation of a transmembrane pore. Furthermore, opsonization of pathogens with complement components (C4b, C3b, iC3b) can target them for phagocytosis by cells expressing complement receptors.

Activation of complement also contributes to inflammation by the release of the anaphylatoxins C3a and C5a. C5a and to a lesser extent C3a trigger release of histamine and other mediators after binding to specific receptors on mast cells or basophils, resulting in vasodilatation. Neutrophils, monocytes, eosinophils and mast cells are attracted to the site of inflammation by C5a, a potent chemoattractant [13, 14]. More recently, it has been established that complement collaborates with the adaptive immune system by augmenting the humoral response to antigens and thereby lowering the threshold for B cell activation [15].

Recent studies have provided evidence for alternative functions of complement that are different from its classical role in the defense against non-self. Compelling evidence suggests that complement components have an effect on 'self', by inducing apoptosis or by triggering cell activation and proliferation. Moreover, complement components can recognize and bind apoptotic cells, and thereby contribute to the efficient clearance of cellular debris.

The Recognition Molecules of the Complement System

C1q

The recognition element of the classical complement pathway, C1q, contains a collagen-like tail region to which the serine proteases C1r and C1s are bound, connected to a globular head region, responsible for ligand binding. The classical complement pathway is activated via the binding of C1q to an activator, resulting in activation of C1r and C1s. Activated C1s subsequently cleaves C4 and C2, resulting in the classical C3 convertase C4b2a. C1q consists of trimeric polypeptide chains, designated A, B and C, and is connected by disulfide bridges between six A-B and three C-C chains, forming six A-B-C units. In the circulation, C1q is predominantly present in the C1 complex, together with a tetramer of two C1r and two C1s molecules. During inflammation or upon initiation of the

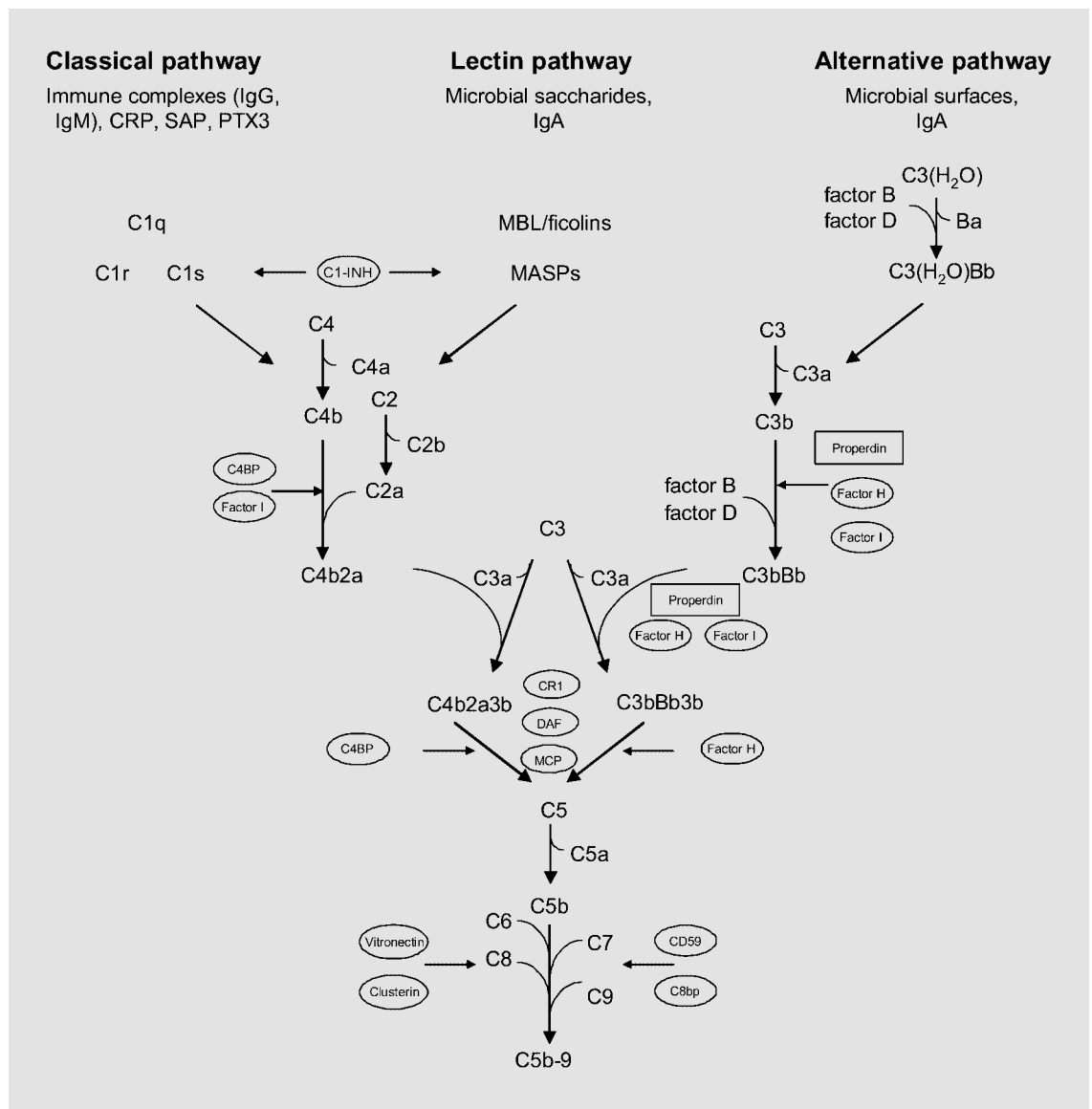


Fig. 1. The classical pathway is activated by the binding of C1q to antibody-antigen complexes, or to other structures, such as pentraxins or apoptotic cells, resulting in the formation of the classical C3 convertase C4b2a. Subsequently, C4b2a cleaves C3 to C3b to form the classical C5 convertase C4b2a3b, which is able to initiate the terminal complement pathway. Activation of the lectin pathway is initiated by MBL and ficolins, recognizing microbial saccharides. Downstream activation of the lectin pathway is almost identical to the classical pathway activation and results in the formation of the same C3 and C5 convertases. The alternative pathway of complement is activated following spontaneous hydrolysis of the thioester bond of native C3, resulting into binding of factor B, which is cleaved

by factor D. This results into generation of C3(H₂O)Bb and, subsequently, of the efficient alternative pathway C3 convertase C3bBb. After stabilization by properdin, an intrinsic amplification mechanism at the level of C3 is provided leading to the formation of the C5 convertase C3bBb3b that finally initiates the terminal complement pathway. This pathway is initiated by the cleavage of C5 into C5a and C5b by C5 convertase. The assembly of C5b with C6 and C7 results in the formation of a hydrophobic C5b67 complex able to insert into lipid membranes. After binding of C8 and one or more C9 molecules to C5b67 a transmembrane pore is formed, called the membrane attack complex. Regulators of complement activation are indicated in ovals.

classical pathway, levels of noncomplexed C1q are increased [16]. The exposed collagen-like domain of C1q is able to interact with cell surface proteins expressed by various cell types. Several C1q-binding cell membrane proteins have been identified. A cell surface protein that binds to the collagen-like region of C1q was designated cC1qR, which appears to be identical to calreticulin (CRT) [17]. Although calreticulin is predominantly an intracellular protein, a recent study proposed that calreticulin can be transported from the endoplasmic reticulum (ER) to the cell surface as a result of the fusion of the ER with the plasma membrane [18]. Substantial evidence has been provided for a role of cC1qR/CRT present on the cell surface in association with CD91 in the clearance of complement-opsonized apoptotic cells [19, 20]. C1qRp was originally described as a putative C1q receptor expressed mainly by monocytes and macrophages, which enhances phagocytosis [21]. However, recent data show that C1qRp is identical to CD93. This strongly suggest that C1qRp does not bind C1q [22]. A distinct C1q receptor that triggers neutrophils to release superoxide was designated C1qRO₂ [23], but the identity of this receptor is not defined. Complement receptor 1 (CR1 or CD35) present on leukocytes and erythrocytes is known to play a role in the recognition of immune complexes opsonized with the activated complement fragments C3b and C4b, leading to their clearance in the liver and spleen.

Evidence suggests that CR1 also binds to C1q via the collagen-like region [24]. The interaction between CR1 and C1q may play a role in immune complex clearance. In addition to C1q-binding proteins that recognize the collagen-like region of C1q, also a receptor for the globular heads of C1q has been characterized and designated gC1qR [25]. Because gC1qR is identical to the mitochondrial protein p32 and p32 believed to be primarily present in intracellular vesicles [26], it has been proposed to rename 'gC1qR' to 'gC1qBP', a binding protein for the globular heads of C1q [27]. The interaction of C1q with cell-surface proteins present on various cell types can trigger an array of cellular responses, including phagocytosis, enhanced microbial killing by phagocytes, induction of chemotaxis, stimulation of immunoglobulin secretion [28] and production of cytokines and chemokines [29].

C1q has been implicated in a number of functions involved in host defense and protection against autoimmunity. The importance of C1q in vivo is demonstrated in C1q-deficient individuals, who are strongly predisposed to the development of the autoimmune disease systemic lupus erythematosus (SLE). More than 90% of individuals with homozygous C1q deficiency develop a SLE-

like illness [30]. Similarly, in mice with deficiencies in C1q, multiple apoptotic bodies are present in the kidney associated with the spontaneous development of autoantibodies and glomerulonephritis [31]. This correlates well with the hypothesis that apoptotic cells, when not efficiently cleared, may represent a source of autoantigens, resulting in an autoimmune response. The role of C1q in the clearance of apoptotic cells is supported by in vitro studies demonstrating that C1q binds directly and specifically to apoptotic cells [32–34], and that opsonization of apoptotic cells with C1q facilitates their uptake by phagocytes [19].

The increased susceptibility of C1q-deficient mice to bacterial infection supports an important role for C1q and the classical pathway of complement in the host defense against pathogens [35]. C1q may affect host defense by its interaction with antibodies, but also by directly binding pathogenic bacteria [36] and HIV [37] resulting in either complement activation or enhanced phagocytosis. C1q can bind to a variety of proteins in an antibody-independent manner, thereby contributing to an inflammatory response seen in for example Alzheimer disease, by binding β -amyloid peptides [38], or to the pathogenesis of prion diseases, by opsonization of prions [39].

Mannose-Binding Lectin and Collectins

Mannose-binding lectin (MBL) is a member of the collectin family of proteins, which are characterized by the presence of both a collagen-like region and a C-type lectin domain. Collectins are structurally related to C1q. MBL binds to a wide array of carbohydrate structures on microbial surfaces and subsequently activates the complement system. MBL can be associated with three MBL-associated serine proteases (MASPs), MASP-1 [40], MASP-2 [41], MASP-3 [42], and a truncated version of MASP-2 called MAP 19 [43]. The MASPs show a similar domain structure, shared also with C1r and C1s. From the MASPs, MASP-2 appears to be the major complement-activating enzyme, upon binding of MBL-MASP complexes to a sugar-rich microbial surface, MASP2 specifically cleaves both C4 and C2, leading to formation of the C3 convertase C4b2a, [44, 45]. The biological function of MASP-1, MASP-3 and MAP 19 has not yet been resolved. Although MASP-1 has been reported to cleave C3, its physiological role is debated [46]. Moreover, MBL can also act as an opsonin and enhance internalization of microorganisms by phagocytic cells carrying the receptor for MBL [47]. The cell surface C1q-binding proteins CRT (cC1qR) [48] and complement receptor 1 (CR1) [49] have been defined as cellular receptors for MBL.

The gene encoding the MBL protein is characterized by several polymorphic sites, both in the promoter region and in exon 1 that can affect the polymerization and secretion of the protein [50], resulting in reduced serum levels of MBL. MBL deficiency appears to be one of the most common immunodeficiencies, and several studies have established an association between MBL deficiency and the susceptibility of an individual to infectious diseases [51, 52]. In addition, MBL deficiency leads to accelerated progression of several chronic diseases such as rheumatoid arthritis [53], cystic fibrosis [54] and SLE [55].

The lung surfactants protein A (SP-A) and protein D (SP-D) are also members of the collectin family of proteins. SP-A and SP-D play an important role in pulmonary host defense. Like MBL, SP-A and SP-D also bind to various microorganisms via their lectin domains and stimulate their removal through opsonin-mediated phagocytosis [56]. However, SP-A and SP-D are not able to activate complement [57]. Mice deficient in SP-A or SP-D are more susceptible to bacterial and viral infections in the lung [58, 59]. Furthermore, recent studies demonstrated the importance of SP-A and SP-D in the pulmonary clearance of apoptotic cells and have shown that both lung surfactants can bind apoptotic cells and promote their ingestion by phagocytes via a receptor complex of calreticulin and CD91 [20]. Based on the capacity of SP-A to bind C1q [60], SP-A can also enhance the clearance of foreign particles in concert with complement [61]. Furthermore, the interaction between SP-A and C1q downregulates the activation of the complement system, thereby minimizing complement-mediated inflammation and tissue damage [61].

Ficolins

Ficolins represent another important group of pattern recognition molecules that are structurally related to the collectins. Ficolins also bind to sugar residues on the surface of microorganisms; however they possess a different type of lectin domain, called the fibrinogen-like domain [62]. Three members of the ficolin family have been characterized: H-ficolin also called Hakata antigen [63], L-ficolin also known as p35 [64], and M-ficolin [65]. Ficolins have been reported to promote the phagocytosis of pathogens through opsonization of microbes [65]. Furthermore, ficolins can contribute to host defense by activation of the lectin pathway, via a mechanism similar to that used by MBL [66, 67]. Both L-ficolin and H-ficolin are associated with MASPs and MASP19 and this complex has the capacity to cleave C4 and C2, resulting in the generation of the C3 convertase C4b2a.

The Effector Phase of the Complement System

The Opsonins C3b and C4b

Activation of complement leading to the proteolytic cleavage of C4 and C3 to C4b and C3b, respectively, is associated with the formation of covalent linkages between C4b and C3b and the activating surface. Both opsonins and their cleavage products, in case of C3b, can serve as ligands for complement receptors. C4b and C3b can interact with CR1 (CD35), while the cleavage product of C3b, iC3b is a ligand for CR2 (CD21), CR3 (CD11b/CD18) and CR4 (CD11c/CD18). These interactions promote immune adherence, phagocytosis, B cell activation, and antigen uptake, processing and presentation [68]. The deposition of C4b and C3b on apoptotic cells is shown to increase the efficiency of their uptake by phagocytic cells [69, 70].

The Terminal Complement Complex C5b-9

The terminal complement pathway involves assembly of C5b with C6, C7 and C8, and polymerization of C9, resulting in the formation of the MAC that functions as a transmembrane pore. The classical view on the action of C5b-9 is that its insertion into cell membranes leads to a direct loss of membrane integrity, resulting in cell death by necrosis [71]. Nucleated cells are relatively resistant to the lytic effects of complement due to expression of complement regulatory proteins and the capacity to remove C5b-9 from the cell surface [72].

Direct lysis of nucleated cells is therefore not a common phenomenon under physiological conditions. Insertion of C5b-9 into nucleated cells can also cause non-lytic effects. C5b-9 has been implicated in cell survival, either by inhibition of apoptosis [73, 74] or protection against lysis [75]. Moreover, C5b-9 can lead to cell activation, with the production and release of inflammatory mediators such as cytokines, prostaglandins, thromboxanes, leukotrienes, and reactive oxygen species [76–78] or by increasing the expression of adhesion molecules [79]. In various cells, C5b-9 can induce DNA synthesis [80, 81] and stimulate cell proliferation [82, 83]. In other circumstances, C5b-9 has been implicated as a trigger to apoptosis. Inhibition of complement activation with an antibody against C5 strongly abrogated the occurrence of apoptosis after ischemia/reperfusion injury [11, 84]. In rat models of human immune-mediated mesangial proliferative glomerulonephritis, the massive increase of apoptosis was dependent on the presence of the terminal complement pathway [85]. The differential effects of C5b-9 are clearly dose-dependent, lytic amounts of C5b-9 will result in

necrosis, whereas sublytic amounts of C5b-9 may lead to cell activation, proliferation and either resistance to, or induction of, apoptosis.

Apoptosis

Apoptosis, or programmed cell death, is an essential process during development and in the maintenance of tissue homeostasis [86]. Cells undergoing apoptosis are characterized by biochemical and morphological changes, including early changes in the distribution of membrane lipids, membrane blebbing, condensation of both cytoplasm and chromatin, and fragmentation of the nucleus and cellular constituents into membrane-enclosed apoptotic bodies [87]. Phagocytosis is the final common end stage of cells undergoing apoptosis. Another mode of cell death is necrosis, characterized morphologically by swelling of the cell, and finally the rupture of the plasma membrane. In contrast to apoptosis, necrotic cell death is a passive process without nuclear changes.

The process of apoptosis can be divided into four distinct phases: initiation, control and integration, execution, and removal of dead cells (fig. 2). Apoptosis can be initiated by many different stimuli including growth factor withdrawal, UV- or γ -irradiation, chemotherapeutic agents, or by a family of transmembrane proteins called death receptors. Specific proteins determine whether the initiation signal is strong enough to justify the commitment of a cell to proceed into the cell death process. The bcl-2 family of proteins, containing both pro- and anti-apoptotic members, regulate the permeability of the mitochondrion [88].

The mitochondria play an important role as stress sensors and determine the 'point of no return'. Upon stress signals, the permeability of the mitochondria is increased, leading to the release of cytochrome c from the mitochondrial membrane into the cytosol where it can initiate the proteolytic cascade observed in apoptosis. Caspases, cysteine proteases belonging to the interleukin-1-converting enzyme/CED-3 family, are the central executioners in the process of apoptosis. The caspase family can be divided into initiator caspases, functioning as signaling molecules, and execution caspases that account for the morphological features of apoptosis. Recent data indicate that apoptosis can occur in the complete absence of caspases [89].

The characteristic changes in the plasma membrane occurring during the apoptotic process enable the recognition and removal of apoptotic cells by phagocytes. The most characteristic change of the cell surface associated with apoptosis is loss of phospholipid asymmetry and exposure of phosphatidylserine. Another change of the

cell surface is the alteration of carbohydrate groups on the membrane of dying cells [90, 91]. Clearance of apoptotic cells can be achieved by professional phagocytes, such as macrophages and dendritic cells, or neighboring semi-professional cells, including fibroblasts, epithelial cells, and mesangial cells.

Defects in the apoptotic cell death process, leading to inappropriate cell death or survival, or disturbances in the clearance of apoptotic cells, are thought to be involved in various chronic diseases, including neurodegenerative disorders [92], autoimmune diseases [93, 94], and cancer [95].

Pentraxins

Pentraxins are acute-phase proteins, usually characterized by a cyclic pentameric structure, that show a strong inter-species homology [96]. The blood levels of these proteins can increase dramatically in response to inflammation, trauma, or infection. The classical short pentraxins, C-reactive protein, and serum amyloid P component (SAP) are produced in the liver in response to inflammatory mediators, most prominently interleukin-6 [97, 98]. Short pentraxins contribute to innate immune responses to microbes, regulation of inflammation, and the scavenging of cellular debris [99, 100]. CRP is considered to be the prototypic acute-phase reactant in man and recognizes microorganisms and dying cells via its pattern recognition capability [101]. The interaction of CRP with C1q can lead to activation of complement via the classical complement pathway [6]. CRP has been shown to have both proinflammatory and anti-inflammatory effects.

CRP can activate monocytes to produce inflammatory cytokines [102] and in vivo injection of CRP enhances tissue damage in an ischemia/reperfusion model via a complement-dependent mechanism [103]. Studies in transgenic mice suggest an important role for CRP in the downregulation of an inflammatory response [104, 105]. CRP inhibits the respiratory burst and chemotaxis of neutrophils [106]. Moreover, CRP can act as an opsonin by binding to microbial organisms and damaged cells [101]. In mice, SAP is a major acute-phase reactant and is the precursor of tissue amyloid P component, a constituent of all types of amyloid [107]. SAP binds to various molecules, including microbial polysaccharides, matrix components, C1q, and to DNA and chromatin. The interaction of SAP with nuclear antigens suggests a role for SAP in their clearance. This hypothesis is supported by findings in SAP-deficient mice that develop anti-nuclear anti-

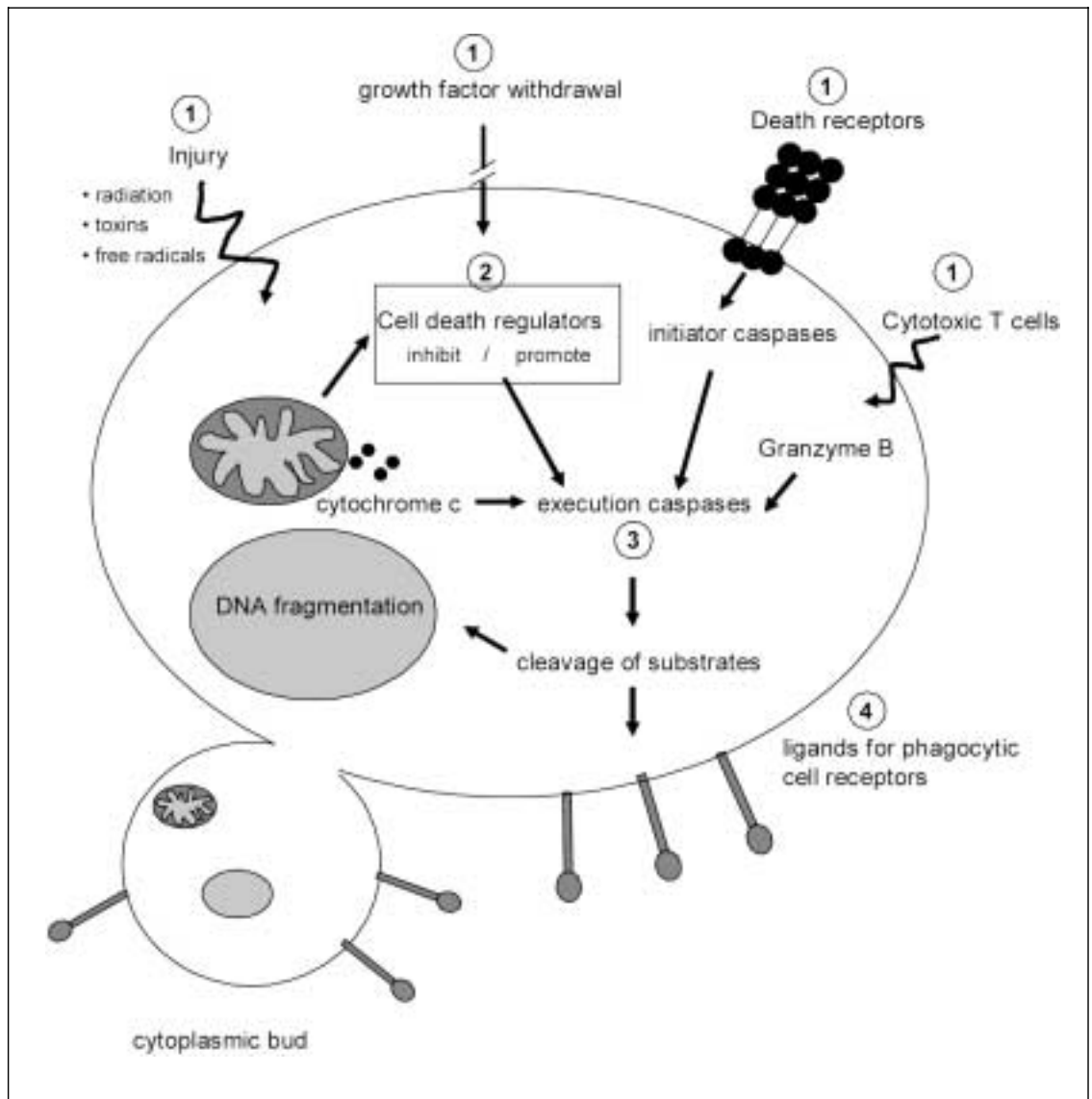


Fig. 2. (1) Apoptosis can be initiated by multiple signals including death receptors, withdrawal of growth factors or injurious agents (e.g. radiation, heat, oxygen radicals). Some stimuli (such as cytotoxic T cells) directly activate execution caspases. Others act by way of initiator caspases, or by mitochondrial events involving cytochrome c. (2) The strength of the apoptosis-inducing signal is evaluated by specific control proteins, which can either inhibit or promote cell death.

(3) If the signal is strong enough, execution caspases are activated that degrade cytoskeletal and nuclear proteins. This results in a cascade of intracellular degradation, including fragmentation of nuclear chromatin, breakdown of the cytoskeleton, and blebbing of the cell membrane, giving rise to the morphological features of apoptosis. (4) The end result is the formation of membrane-enclosed vesicles (apoptotic bodies) and the recognition and engulfment of apoptotic material by phagocytic cells.

bodies and glomerulonephritis [108]. Moreover, SAP has been shown to enhance the clearance of chromatin from the circulation [108, 109]. In contrast to these protective effects of SAP, SAP can also enhance the virulence of some bacteria [110] and participate in the pathogenesis of amyloidosis [111].

The role of SAP and CRP in the amplification of the innate immune response and the regulation of the inflammatory response are suggested to be mediated through Fc receptors for IgG (FcγR). A recent report provided in vivo evidence that CRP can down-regulate inflammatory responses through interaction with FcγR [112]. CRP is

described to bind both to Fc γ R1 (CD64) and Fc γ RIIa (CD32), and that SAP additionally interacts with Fc γ RIII (CD16) [113–115]. However, others question the interpretation of these data and suggest that the binding of pentraxins to Fc γ R is influenced by contamination of CRP with other proteins, as well as by the interference of the Fc part of the detection antibodies with Fc γ R [116, 117].

PTX3 is the first cloned long pentraxin, structurally related to, yet distinct from, classical short pentraxins. Long pentraxins differ from short pentraxins for the presence of an unrelated long N-terminal domain. Inflammatory cytokines can induce the expression of PTX3 in a variety of cells, most prominently endothelial cells and mononuclear phagocytes [118, 119]. PTX3 is suggested to play an important role in the regulation of inflammatory reactions, innate immunity and in the clearance of self-components similar to the short pentraxins SAP and CRP. PTX3 plays an important role in the resistance against pathogens, as demonstrated by studies with PTX3-deficient mice [120]. PTX3 can also bind C1q and it participates in the clearance of apoptotic cells and particular microorganisms [120–122]. Expression of PTX3 in a late phase of certain neurological disorders has been associated with a potential protective activity of PTX3 [123]. Transgenic mice overexpressing the PTX3 gene were found to be more resistant to endotoxic shock and sepsis [124]. Although in these mice the excessive inflammatory response was associated with a more effective control of infection, the augmentation of proinflammatory cytokines in PTX3 transgenic mice resulted in a higher degree of tissue damage and enhanced lethality in a model of ischemia/reperfusion injury [125]. Moreover, PTX3 levels are elevated in a number of human disorders, such as myocardial infarction [126], rheumatoid arthritis [127], and sepsis [128], and in such conditions PTX3 is suggested to contribute to tissue damage by activation of the complement system. PTX3 potentiates the expression of tissue factor on endothelial cells, thereby contributing to thrombogenesis and vascular ischemia [129].

After cloning of PTX3, other long pentraxins were identified, including guinea pig apexin [130], XL-PXN1 from *Xenopus* [131], rat neuronal pentraxin 1 (NP1) [132], human neuronal pentraxin 2 (NPTX2) [133], and neuronal activity regulated pentraxin (NARP) [134]. These members of the long pentraxin family are constitutively expressed in the central nervous system.

The Double-Edged Sword of the Innate Immune System

Activation of the innate immune system can have diverse and opposing effects (fig. 3). On the one hand, molecules of the innate immune system have many protective functions, contributing to host defense against invading pathogens. Moreover, innate molecules are suggested to be involved in the clearance of self antigens. On the other hand, activation of innate immunity can contribute to inflammation and tissue injury and is described to contribute to the pathogenesis of many diseases.

Complement and Pentraxins in the Host Defense against Pathogens

The complement system and members of the pentraxin family have a major role in the host defense against invading pathogens. Detailed knowledge about the contribution of complement components and pentraxins has been obtained from naturally occurring complement deficiencies and by the generation of knockout mice [reviewed in ref. 135]. The absence of several complement components and pentraxins has been associated with recurrent infections [136]. Complement and pentraxins contribute to host defense by killing pathogens directly, targeting pathogens for clearance by phagocytic cells by opsonization, stimulating inflammation by the release of the C5a and C3a, and last but not least, indirectly by enhancing and directing the adaptive immune response.

Role of Pattern Recognition Molecules of the Innate Immune System in the Clearance of Endogenous Material

Complement and pentraxins are also involved in the clearance of cellular debris. The safe clearance of cells dying by apoptosis is essential in order to prevent tissue injury by the release of their noxious contents and self-antigens. If apoptotic cells are not cleared efficiently, they undergo secondary necrosis, resulting in inflammation. Macrophages and dendritic cells can internalize apoptotic cells and present antigens derived from processing of these apoptotic cells to T cells [137, 138]. In case of disturbed clearance of apoptotic cells, danger signals may be provided, converting the response to these antigens from tolerogenic to immunogenic [139]. The interaction of early complement components and pentraxins with apoptot-

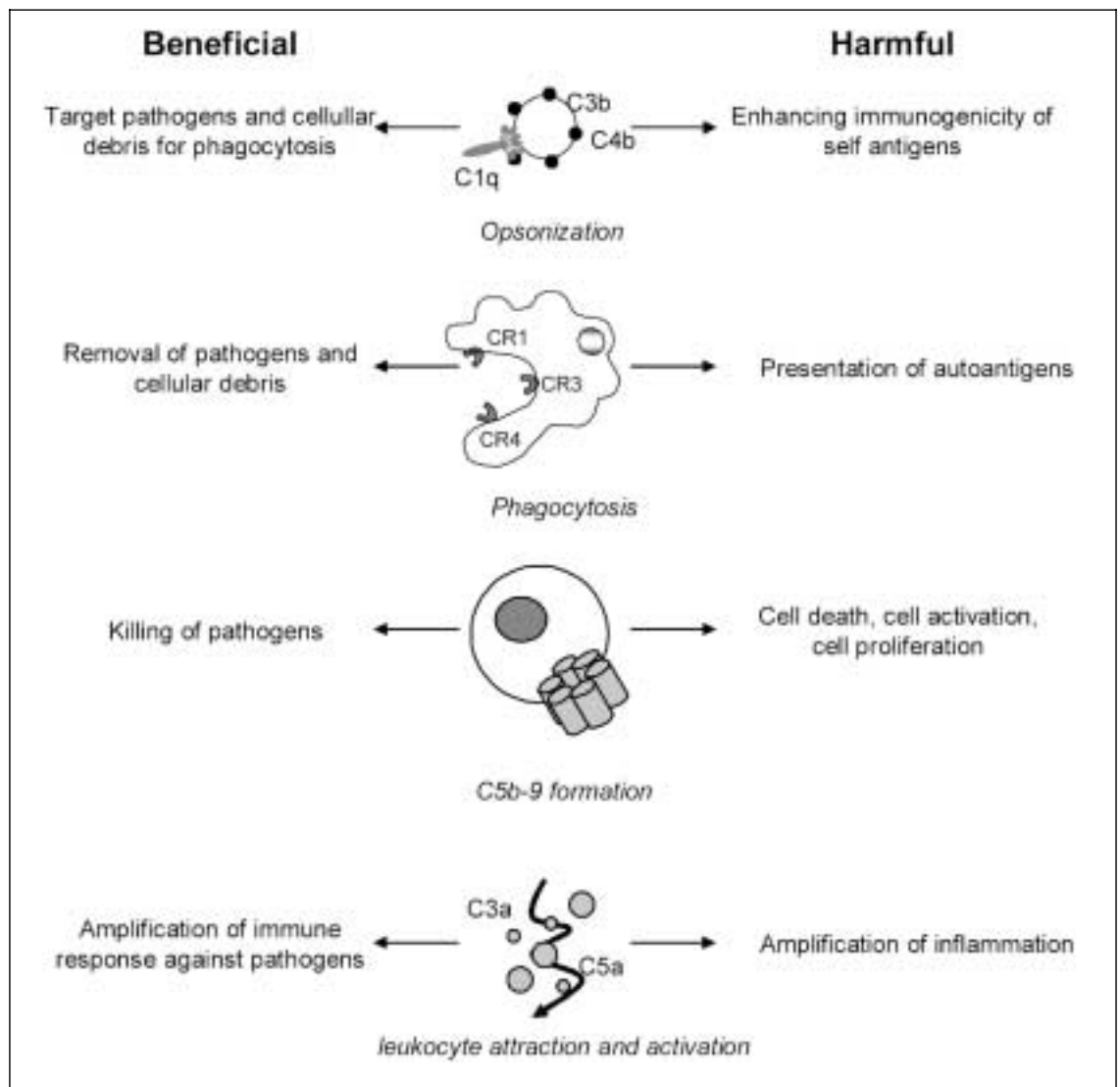


Fig. 3. The effects of complement activation can be both beneficial and harmful for the host. Activation of complement can lead to opsonization of pathogens or cells with molecules such as C3b thereby targeting them for clearance. On the other hand, complement opsonization can potentially enhance the immune response against self-antigens. Complement contributes to the phagocytosis of pathogens and cellular debris that otherwise could cause damage; however, clearance of self-antigens under inflammatory conditions may favor

the development of an autoimmune response. The formation of C5b-9 can contribute to host defense by directly killing target cells, but can also trigger various events in cells, including activation, proliferation and apoptosis, that can cause damage to self. Complement can contribute to the inflammation by attracting and activating leukocytes that aid in the host defense against pathogens, but at the same time can contribute to tissue injury.

ic debris and the increased uptake of apoptotic cells by phagocytes in the presence of complement suggest a role for these molecules in the clearance of dying cells and thereby in the maintenance of tolerance. This hypothesis is further supported by observations that deficiencies in these molecules, both in humans and in mice, predispose to autoimmunity.

The Complement System as an Important Mediator of Inflammatory Tissue Damage

Evidence has been presented that complement components and pentraxins may target its effects to self-tissue via similar mechanisms required for optimal host defense. Thereby, complement and pentraxins can cause tis-

sue damage and inflammation and have been implicated in the pathogenesis of numerous inflammatory disorders, including autoimmune diseases, immune complex diseases, neurodegenerative disorders, allograft rejection, and ischemia/reperfusion injury, as occurring notably in myocardial infarction, stroke, and major surgery. Undesired complement activation is initiated by the binding of recognition molecules of the complement system to damaged cells. Activation of complement results in the formation of several proinflammatory mediators, including the anaphylatoxins C3a, C5a. Furthermore, the membrane attack complex can contribute to inflammation and tissue injury. C5a is a potent chemoattractant for neutrophils, monocytes and eosinophils and both C3a and C5a can stimulate the synthesis of pro-inflammatory mediators. Formation of the terminal complement complex C5b-9 can lead to cell activation, proliferation and cell death by necrosis or apoptosis. Several studies demonstrated the involvement of complement in inflammatory tissue destruction. Inhibition of C5 strongly abrogated apoptosis and inflammation, leading to a significant reduction in renal function loss in a murine model of renal ischemia/reperfusion injury [84]. Mice deficient in C3, C5 and C6 were protected from ischemia/reperfusion injury of the kidney, skeletal muscle, and intestine [140–142]. Furthermore, a recent study demonstrated that the absence of local C3 production promoted renal graft survival [143]. The absence of C3 not only prevented direct complement-mediated injury but also impaired the priming of T cells against donor antigens. Together, these data indicate that the recognition of altered self by complement factors may be beneficial to support clearance and to prevent autoimmunity, but, in case of extensive injury, may also lead to enhanced inflammation and tissue damage.

Therapeutic Strategies to Prevent Undesired Complement Activation

During recent years, various therapeutically active complement inhibitors have been developed to prevent undesired effects of complement activation. Under physiological conditions, several soluble and membrane-associated complement-regulatory molecules effectively control complement activation to protect the host. A number of physiological complement inhibitors have been evaluated for therapeutic application, including C1 inhibitor [144, 145] and soluble complement receptor 1 [144]. In addition, a variety of synthetic compounds have been tested for their capacity to inhibit complement.

The major challenge in the development of therapeutic complement inhibitors is to balance the beneficial effects of complement inhibition with the preservation of sufficient functional activity for the defense against pathogens and clearance of self-antigens.

Concluding Remarks

The complement system is a potent defense machinery against infection. However, complement activation can lead to injury of tissue during inflammatory processes. At the same time biologically active components and fragments of complement molecules can induce enhancement of innate immune function and initiate the acquired immune response. Following recruitment of the acquired immune response, complement together with antibodies and immune cells, composes a potent mechanism of enhanced defense. More recently, complement together with other molecules of innate defense has been implicated in the induction of apoptosis and their removal from injured sites. In addition complement seems to play a crucial role in the regulation of immune responses, and it is a challenge for the future to determine how complement regulates immune responses or controls tolerance induction.

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