Complement and Its Receptors: New Insights into Human Disease

V. Michael Holers

Department of Medicine, University of Colorado School of Medicine, Aurora, Colorado 80045; email: michael.holers@ucdenver.edu

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Abstract

Although new activation and regulatory mechanisms are still being identified, the basic architecture of the complement system has been known for decades. Two major roles of complement are to control certain bacterial infections and to promote clearance of apoptotic cells. In addition, although inappropriate complement activation has long been proposed to cause tissue damage in human inflammatory and autoimmune diseases, whether this is indeed true has been uncertain. However, recent studies in humans, especially those using newly available biological therapeutics, have now clearly demonstrated the pathophysiologic importance of the complement system in several rare diseases. Beyond these conditions, recent genetic studies have strongly supported an injurious role for complement in a wide array of human inflammatory, degenerative, and autoimmune diseases. This review includes an overview of complement activation, regulatory, and effector mechanisms. It then focuses on new understandings gained from genetic studies, ex vivo analyses, therapeutic trials, and animal models as well as on new research opportunities.

INTRODUCTION

The complement system is an ancient member of the immune system and consists of multiple classes of components. The system includes a large number of soluble proteins that are found in the circulation and in tissues and that form the basis for the presence of three activation pathways: the classical, alternative, and lectin (**Figure 1**). Additionally, multiprotein membrane-spanning complexes form, and membrane-bound receptors engage bioactive effector fragments, and both processes exert substantial biological effects. Both soluble and cell-associated regulatory proteins serve to modulate activation or propagation of the system at several steps during the activation process.

A wide variety of important protective roles have been ascribed to the complement system, including recognition and clearance of foreign pathogens and antigens (1), phagocytosis of opsonized targets (2), promotion of humoral immune responses (3), modulation of cellular immune responses (4), noninflammatory clearance of self-antigens derived from apoptotic processes (5), and immune complex transport (6). Also suggested by recent studies, but less well appreciated, are the hypotheses that complement promotes the autoinflammatory response to injured self-tissue following recognition of neoepitopes by natural antibodies (7); that it indirectly regulates the growth of inflammatory tumors (8); that it shapes the composition of the natural antibody repertoire (9); and, perhaps most unexpectedly, that it promotes the enhancement of tissue regeneration following

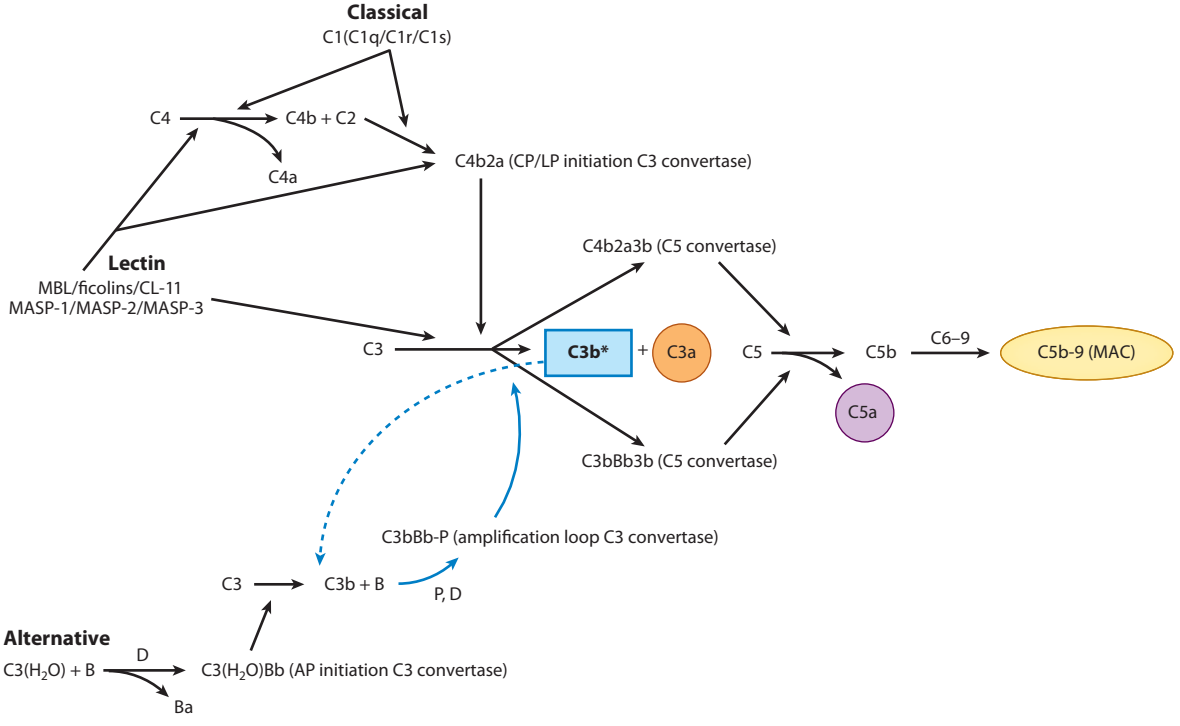

Figure 1

Overview of complement initiation and effector pathways. The complement system can be initiated through the classical (CP), lectin (LP), or alternative (AP) pathways, resulting in the formation of C3 convertases that cleave and activate this protein to the C3b and C3a forms. Subsequent activation of C5 and further components of the complement cascade results in formation of the membrane attack complex (MAC) and C5a. All three initiator pathways can result in the generation of C3b, which initiates the amplification loop when Factor B binds C3b and is cleaved by Factor D (*dotted line*). This process greatly increases the rate at which effector molecules are generated. (Abbreviations: MASP, MBL and associated proteases; MBL, mannose-binding lectin; *indicates covalent attachment.)

transplantation-induced injury in the liver (10) and the neovascularization that is associated with neonatal oxygen-induced injury (11). Finally, although the complement system was historically considered to be a component of the innate immune system, its capacity to modulate and direct humoral and cellular immune responses now places it more properly at the interface between innate and adaptive immunity (1). Additionally, the recently recognized roles of complement in homeostasis point to several nontraditional functions that bridge immunity and developmental biology (12).

An important clinically relevant concept, explored herein, is that, when inappropriately directed to recognize and attack self-tissues, the complement system can damage target organs and play pathophysiologically important roles in human autoimmune and inflammatory diseases. In such cases, the powerful effector mechanisms of complement that should be focused on recognition and clearance of foreign pathogens and antigens instead appear to be directed to self-tissues and cause tissue damage. This process is now well established as key to the pathophysiology of several human diseases where the presence of dysfunctional regulatory proteins due to rare genetic polymorphisms or mutations allows excessive complement activation in systemic thrombotic microangiopathies such as atypical hemolytic uremic syndrome (aHUS) (13, 14) and in hemolytic disorders such as paroxysmal nocturnal hemoglobinuria (PNH) (15). Autoimmune diseases such as the rare disorder neuromyelitis optica (NMO), which is associated with pathogenic IgG anti-aquaporin-4 autoantibodies, are also associated with local complement activation (16). Importantly, as discussed below, we know these are complement-dependent disorders because treating them with the complement-inhibitory anti-C5 monoclonal antibody eculizumab is highly effective. In addition, because complement sits at a key interface in the immune system, its therapeutic modulation could be effective in controlling the inappropriate pathogenic activation of both innate inflammation and adaptive immune responses in many common disorders beyond these rare conditions (17, 18).

Insights into additional important roles for this system have been provided by careful studies of complete and partial deficiency states of complement activation proteins. For example, complete deficiencies of complement pathway components are strongly associated with the development of bacterial infections (19). In this regard, pathogens demonstrate many different mechanisms that attempt to evade the complement pathway. Although I do not focus here on this broad area, interested readers are directed to a recent review for an introduction to the topic (20). Additionally, early classical pathway deficiencies are associated with systemic lupus erythematosus (SLE)-like syndromes that appear to be due to inefficient clearance of apoptotic self-tissues and a lack of tolerance to certain self-antigens (5).

COMPLEMENT ACTIVATION MECHANISMS AND PATHWAYS

The classical complement pathway is initiated by antibody-dependent as well as antibody-independent mechanisms (21) (**Table 1**). With regard to human antibodies, the general order of complement-fixing potential is IgM > IgG3 > IgG1 > IgG2 >> IgG4. IgA can activate the alternative pathway, whereas IgE is not an effective complement activating isotype except under unusual circumstances. The mechanism of activation exhibited by IgM involves a conformational change described as "stapling down" that occurs after it binds to a multimeric antigen. Activation of IgG isotypes engages the classical pathway when they bind antigen in a form that allows the classical pathway component C1q to bind at least two, and perhaps as many as six, closely approximated IgG molecules (21). C1r then undergoes autocatalytic activation, and C1s is transactivated. As a result, C1s sequentially cleaves the classical pathway proteins C4 and C2 into C4a and C2b, respectively (**Table 2**), and forms the multiprotein complex C4b2a that serves as the classical

Table 1 Complement activators that can initiate the pathway with which they are associated

Pathways		
Classical	**Alternative**	**Lectin**
Immune complexes (IgM, IgG)	"Tick-over"	Repeating simple sugars
C-reactive protein	Amplification pathway	G0 carbohydrate glycoforms
Apoptotic bodies	Endotoxin	Cytokeratin-1
Beta-amyloid fibrils	IgA immune complexes	
Serum amyloid P	Polysaccharides	
Mitochondrial products	C3 nephritic factor	
C4 nephritic factor		
PMX3		

pathway C3 convertase activating enzyme (**Figure 1**). Antibody-independent activators include the pentraxin C-reactive protein, beta amyloid fibrils, serum amyloid P, and tissue-damage products such as mitochondrial membrane proteins, each of which directly binds C1q in a manner that allows subsequent complement activation. Another pentraxin, PMX3, also interacts with C1q as well as other complement activation components including mannose-binding lectin (MBL), ficolins 1 and 2, and the regulatory proteins Factor H and C4-binding protein. For both C-reactive protein and

Table 2 Complement activation pathway proteins

Component	Approximate serum concentration (μg/ml)	Approximate molecular weight
Classical pathway		
C1q	70	410,000
C1r	34	170,000
C1s	31	85,000
C4	600	206,000
C2	25	117,000
Alternative pathway		
Factor D	1	24,000
C3	1,300	195,000
Factor B	200	95,000
Lectin pathway		
MBL	150 (very wide range)	600,000
MASP-1	6.0	83,000
MASP-2	0.5	76,000
MASP-3	–	95,000
Membrane attack complex (MAC)		
C5	80	180,000
C6	60	128,000
C7	55	120,000
C8	65	150,000
C9	60	79,000

PMX3, the amplification of downstream activation during infection is decreased while pathogen clearance is promoted (22).

The alternative pathway does not require specific activation. Instead, in a process termed "tick-over," C3 undergoes conformational changes and spontaneous assembly, which results in its activation through the serial activities of its components Factor B and Factor D (**Table 2**). This process is also associated with stabilization by properdin to form the C3 convertase C3bBbP (23). Recent studies have suggested that tick-over is accelerated through the binding of native C3 to surfaces including gas bubbles, platelet surfaces, biomaterials, and microparticles (24). In addition, repeating polysaccharides, endotoxin, IgA-containing immune complexes, the stabilizing autoantibody designated C3 nephritic factor, and some immunoglobulin light chains serve to promote alternative pathway activation.

Components of the alternative pathway also serve as an "amplification loop" (**Figure 1**) so that C3b, which is activated and deposited on a target by any pathway, binds Factor B; these components also initiate further cleavage events (25). On the basis of in vitro hemolytic assays focusing on the classical pathway, the alternative pathway amplification loop was long thought to be a relatively minor contributor to tissue inflammation. However, over the past several years, it has been shown to be one of the most essential mechanisms for the full elaboration of complement-dependent injury in vivo (26, 27).

The lectin pathway is initiated by target recognition through MBL, ficolins 1–3, and CL-11 (**Table 2**). All these protein components belong to a family of collectins that are involved in the recognition and clearance of foreign organisms such as bacteria and viruses (28–30). In addition to these protective roles, the lectin pathway may be engaged during tissue injury through the direct recognition of self-proteins such as cytokeratin-1 (31) as well as through autoantibodies containing agalactosyl (G0) carbohydrates that bind MBL and are important in antibody-driven injury processes (28–30). The lectin pathway may also be involved in the activity of certain pathogenic IgM natural antibodies (32). After recognition, initiation proceeds through the activities of processes mediated by MASP-1, MASP-2, and MASP-3, resulting in cleavage and activation of C4, C2, and then C3 in the same manner as that of the classical pathway C3 convertase (**Figure 1**).

Through these activation processes, multicomponent C3 convertases (activating enzymes) are formed through each pathway, and C3 is subsequently cleaved. During C3 activation, the thioester bond in C3 allows for covalent attachment in the C3b form through ester or amide linkages to other molecules (33). This covalent linkage "marks" the attached target as an immunologically distinct opsonized molecule. Following attachment, C3b is sequentially cleaved to iC3b/C3dg/C3d, thus providing the capacity to interact with specific C3 receptors that bind with high affinity to these fragments (3). Recently, X-ray crystallography with and without other associated proteins was successfully used to determine the structure of C3 and its fragments. Such findings have greatly informed our understanding of this process (34, 35). The activation of C3 is followed by the formation of the multiprotein C5 convertase. This results in the cleavage of C5 to C5b and in assembly of the pore-like membrane attack complex (MAC) through the serial addition of C6–C9. Also generated through this process are the soluble anaphylatoxins C3a and C5a (36).

SITES OF SYNTHESIS AND RELEASE OF COMPLEMENT PROTEINS

It has been long appreciated that the liver is the major site of synthesis of complement activation proteins, several of which are acute-phase proteins. Complement is also synthesized by many other cell types, including monocyte/macrophage, fibroblast, renal mesangial, epithelial, endothelial, lymphoid (both T and B cells), and adipocyte (37). Even though the relative importance of local tissue synthesis is not yet well understood, there is an emerging understanding that locally

Table 3 Complement receptors

Receptors	Approximate molecular weight	Major activities[a]
Complement receptor 1 (CR1, CD35)	190,000–250,000	Immune complex transport (E); phagocytosis (PMN, Mac); immune adherence (E); cofactor and decay-acceleration; secondary Epstein-Barr virus receptor
Complement receptor 2 (CR2, CD21)	145,000	B cell coactivator, primary Epstein-Barr virus receptor, CD23 receptor
Complement receptor 3 (CD11b/CD18)	170,000 (α chain)[b]	Leukocyte adherence, phagocytosis of iC3b-bound particles
Complement receptor 4 (CD11c/CD18)	150,000 (α chain)[b]	Leukocyte adherence
CRIg	45,000	Immune complex/pathogen clearance
C5a receptor (CD88)	50,000	Cell activation, immune polarization, chemotaxis
C5L2	50,000	Modulates C5a function
C3a receptor	75,000	Cell activation

[a]Abbreviations: E, erythrocyte; Mac: macrophage; PMN, neutrophil.
[b]Common 95,000 β chain.

produced complement factors are important in the generation of complement-dependent humoral immune responses (38) and may also be especially important in organ-specific injury. For example, in experimental renal allotransplantation, donor kidney–derived C3 synthesis is important in the development of injury and rejection (39). Not unexpectedly, complement synthesis is also modulated at local sites of inflammation under apparent cytokine-dependent control (40).

COMPLEMENT RECEPTORS

High-affinity complement receptors are engaged by proteolytic cleavage fragments generated from a subset of complement proteins during the activation process (**Table 3**). With regard to activation fragments of C3, five distinct receptors, each with preferential binding for different fragments of C3, have been well characterized (3, 41). Complement receptor type 1 (CR1, CD35) is a widely distributed molecule: It is found on erythrocytes, polymorphonuclear leukocytes, mononuclear phagocytes, B cells, some T cells, and mesangial phagocytes. CR1 binds both the C4b and C3b ligands, which are the initial degradation products of C4 and C3 that are covalently bound to targets following activation. CR1 also acts as a receptor for C1q. CR1 is the major receptor on erythrocytes for binding and processing immune complexes in vivo, whereas on neutrophils and macrophages it acts as a phagocytosis-promoting receptor. On B lymphocytes, CR1 may function both as a processing molecule, converting C3b to iC3b (42), and as a contrasting signal to CR2 by serving as a means to down-modulate B cell responses to C3b-coated antigens (43). Finally, recent studies have shown that CR1 is also a previously unrecognized secondary receptor for Epstein-Barr virus (44).

Following the generation of C3b, the molecule is degraded by Factor I and cofactors such as CR1 into iC3b and C3dg and then into C3d through nonspecific proteases. The latter three forms interact with CR2/CD21, a receptor expressed on B cells, epithelial cells, follicular dendritic cells (FDCs), thymocytes, and a subset of peripheral T cells. CR2 is important in promoting B cell activation by C3 fragment–opsonized antigens and in trapping and retaining immune complexes

on FDCs within lymphoid tissues to sustain immunologic memory (45). Interestingly, CR2 is also the primary receptor for the Epstein-Barr virus as well as for the immunoregulatory molecule CD23, where the interaction promotes the production of IgE.

CR3 and CR4 are β2 integrins composed of two chains: an α chain of unique composition and a common β chain. CR3 and CR4 bind the C3-degradation product iC3b and are expressed on tissue macrophages, mononuclear phagocytes, polymorphonuclear leukocytes, and FDCs. The fifth receptor for C3 fragments is the complement receptor of the immunoglobulin superfamily (CRIg), which is expressed on Kupffer cells, which are liver-resident macrophages. CRIg plays a major role in the clearance of complement-coated particles and large complexes in circulation, which in turn is required for the effective clearance of opsonized infectious organisms (46).

Additional receptors recognize anaphylatoxic peptides released from C5 and C3 during activation. C5a is a 74–amino acid fragment of C5 that is released during C5 activation, and its receptor (C5aR, CD88) is a member of the rhodopsin family (36). C5a demonstrates multiple proinflammatory properties, including leukocyte chemotaxis; aggregation of neutrophils and platelets; release of mast cell mediators; and the generation of leukotrienes, cytokines, and reactive oxygen metabolites. In addition, engagement of C5aR results in important cross talk with IgG Fc receptors. This process sets the threshold for activation by controlling the relative ratio of activating versus inhibitory Fc receptors, which also help to modulate the characteristics of intracellular signaling responses to C5a (47). Although its exact role remains uncertain, C5L2 is a structurally similar molecule without an intracellular signal activating domain whose biologic role appears to be the modulation of C5a functional effects (48).

C3a is another structurally analogous proinflammatory protein derived from C3 that binds the C3a receptor (C3aR) (49). C3aR is widely distributed and is expressed on neutrophils, monocytes/macrophages, mast cells, hepatocytes, bronchial and alveolar epithelial cells, vascular endothelium, and astrocytes, among other cells. Although C3a appears to demonstrate fewer biological effects than C5a, it plays key roles in inflammatory disorders such as asthma (50) and ischemia-reperfusion injury in the kidney (51).

REGULATORS OF COMPLEMENT ACTIVATION

Befitting its potential for injury of self-tissues, complement is a tightly regulated system with modulators that act at many steps of the pathway (52) (**Table 4**). **Figure 2** illustrates points during complement activation at which natural inhibitors act upon and modulate the complement pathway. Specific molecular mechanisms that are utilized to block complement activation include (*a*) inhibiting activating proteases such as C1s, (*b*) acting as a competitive inhibitor/dissociation factor for multicomponent enzyme complexes, (*c*) performing necessary cofactor roles in proteolytic cleavage, and (*d*) working as a substrate-specific protease. Because of the presence of these many inhibitors, the relative activation state of the complement system at any site can be considered to reflect the relative local balance of these two opposing forces.

With regard to specific regulatory proteins, the classical and lectin pathways are both blocked by C1-inhibitor (C1-INH) (53). C1-INH is a member of the serine proteinase inhibitor (serpin) family and serves as an irreversible trap for C1r/C1s and MASP-1/MASP-2. In addition, C1-INH demonstrates noncomplement activities: It inactivates the proteases kallikrein, Factor XIa, Factor XIIa, and plasmin of the contact and clotting systems.

As noted above, the C3/C5 activation steps are major convergence points in the complement pathway. Not unexpectedly, a relatively large number of proteins serve to modulate these steps. For example, the C3 and C5 convertases undergo a spontaneous decay in which the proteins are no longer physically associated in a multiprotein complex. A set of proteins then serves to accelerate

Table 4 Complement regulatory proteins[a]

Soluble regulatory proteins	Approximate serum concentration (μg/ml)	Approximate molecular weight	Major functions
Positive regulation			
Properdin	25	220,000	Stabilizes alternative pathway C3/C5 convertases
Negative regulation			
C1-INH	200	105,000	Inhibits C1r/C1s, MASPs
C4-bp	250	550,000	Inhibition of classical pathway C4b2a C3 convertase by decay acceleration and cofactor activity for C4b cleavage by Factor I
Factor H	500	150,000	Decay-acceleration; inhibition of alternative pathway C3 convertase by decay-acceleration and cofactor activity for C3b cleavage by Factor I
Factor I	34	90,000	Cleavage of C3b/C4b
Anaphylatoxin inactivator (carboxypeptidase)	35	280,000	Generates C3a/C5a desArg
S protein (vitronectin)	500	80,000	Blocks MAC formation
SP-40,40 (clusterin)	60	80,000	Blocks MAC formation
Membrane regulatory proteins			
Decay-accelerating factor (CD55)		70,000	Inhibition by decay acceleration of the classical and alternative pathway C3 convertases
Membrane cofactor protein (MCP, also known as CD46)		45,000–70,000	Inhibition by cofactor activity for classical and alternative pathway proteins C4b and C3b, respectively
CD59		20,000	Blocks C8-C9 and C9
CRIT		30,000	Blocks C2
SUSD4		30,000	Multipathway inhibitor

[a]Abbreviations: CRIT, C2 receptor inhibitor trispanning; MAC, membrane attack complex; SUSD4, sushi domain-containing protein 4.

the normal decay of the C3 and C5 convertases. C3b may also be irreversibly inactivated by using Factor I (i.e., C3b inactivator) to cleave it to iC3b, which cannot support further activation. Factor I cleaves C3b and C4b at specific sites when they are either free in the fluid phase or target bound.

In the fluid phase, two major proteins block complement activity at the C3/C5 activation steps: Factor H and C4b-binding protein (C4-bp). Factor H serves as a decay accelerator of the normally slow spontaneous decay of Bb from C3b in the alternative pathway C3 convertase process and of Bb from C3(H_2O) in the tick-over process. In addition, Factor H exhibits strong cofactor activity for Factor I–mediated cleavage of C3b into iC3b and weak activity for cleavage of C3(H_2O) to iC3(H_2O). Factor H consists of 20 repeating units designated short consensus repeats (SCR, also known as complement control protein repeats); as such, it belongs to an SCR-containing gene family (54). In addition to fluid-phase activity, Factor H binds to target sites on tissues and cells through a process requiring interactions by SCRs 19–20. Ex vivo studies also suggest the involvement of other functional domains that play important roles in mediating surface binding (55–57) with tissues that express both polyanions and fixed C3b/C3d. Factor H is a complex molecule that circulates partly in dimers and tetramers, which may be the most functionally important conformation owing to the increased avidity of these forms (56, 58). This

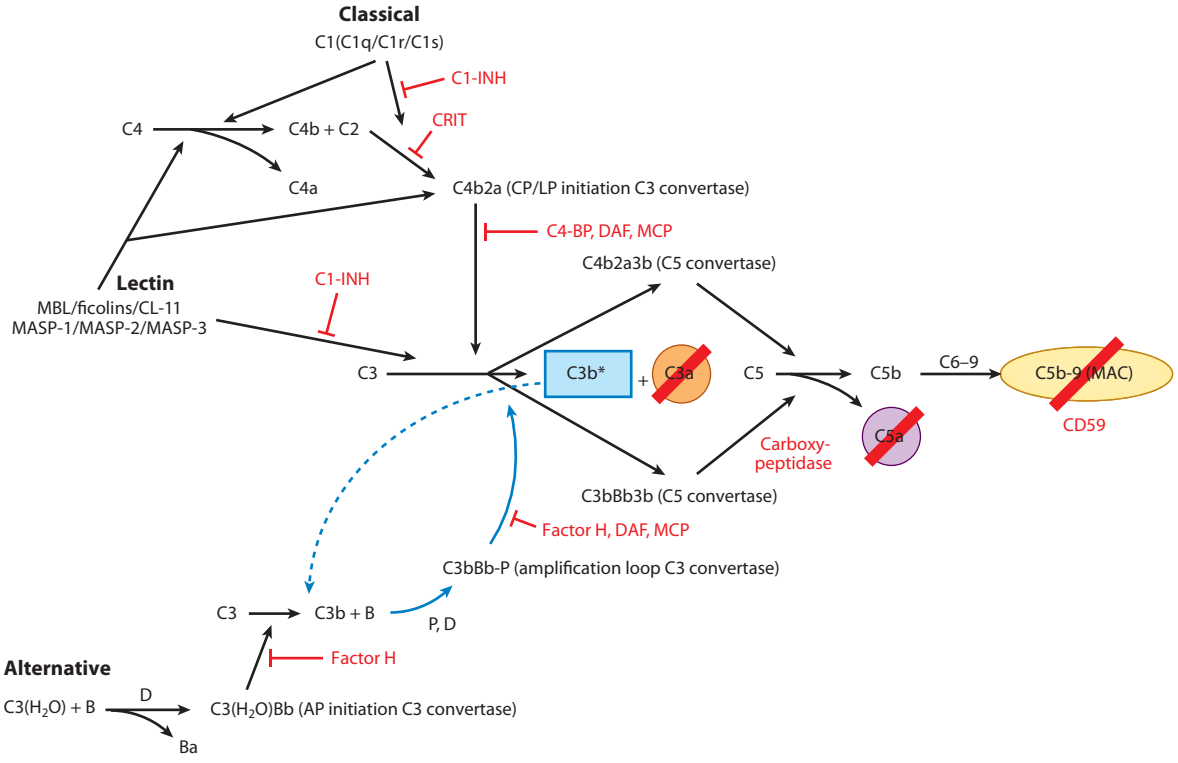

Figure 2

Sites of inhibitory activity manifest by natural complement regulatory proteins. Many points in the complement pathway are subject to regulatory mechanisms, as indicated by the points shown. The rate of generation of effector molecules reflects the balance between activation and inhibitory mechanisms. [Abbreviations: C1-INH, C1 inhibitor; C4-BP; C4b-binding protein; CRIT, C2 receptor inhibitor trispanning; DAF, decay-accelerating factor; MAC, membrane attack complex; MASP, MBL and associated proteases; MBL, mannose-binding lectin; MCP, membrane cofactor protein (also known as CD46); *indicates covalent attachment.]

binding process is necessary for the elaboration of the protective complement alternative pathway involving C3 convertase inhibitory function on the tissue or cell surface. The binding of Factor H to acellular surfaces such as the basement membrane is now thought to be a necessary and major protective role of this molecule (14).

The alternative pathway is also positively regulated by the multimeric protein properdin (59). As noted above, properdin is a protein that can bind to both C3b and Factor B in the C3 convertase and keeps these proteins together and active by resisting decay acceleration. Recent studies have also suggested that properdin can recognize certain target tissues on pathogens as well as self-tissues and can help to initiate activation of the alternative pathway (59).

Within the classical pathway, C4-bp is a fluid-phase protein that exhibits regulatory activities similar to those of Factor H but directed toward C4b rather than C3b (60). C4-bp inactivates C4b by serving as a cofactor for Factor I–mediated cleavage, and it accelerates the spontaneous decay of C2a from C4b. C4-bp also exhibits both of the functional characteristics of Factor H: cofactor activity and decay acceleration.

Additional soluble proteins that inhibit the assembly or membrane insertion of the MAC (C5b-9) have also been identified. These proteins include SP-40,40 (clusterin) and S protein (vitronectin). S protein is identical to vitronectin (serum spreading factor) and is a widely distributed

protein in the intercellular matrix. S protein may act by incorporating itself into the C5b-7 complex that is then converted into a hydrophilic, nonmembrane-binding inactive complex. The complement regulatory activity of SP-40,40 is similar to that described for S protein.

Several additional proteins on the cell membrane exhibit similar functions designed to block complement activation. These proteins include DAF (decay-accelerating factor; CD55), MCP (membrane cofactor protein; CD46), and MIRL (membrane inhibitor of reactive lysis; CD59). DAF is an approximately 70-kDa glycoprotein that acts by binding C3b or C4b on the cell membrane and increasing the spontaneous decay of both the classical and alternative pathway C3 complexes (52). DAF is a widely distributed membrane protein that has a glycophosphatidylinositol anchor. CD46 is another widely distributed regulatory protein that acts as a required cofactor on the cell membrane for the cleavage of C3 or C4 into their inactive forms, iC3b and iC4b (52). Similar to DAF and Factor H, CD46 protects cells from inadvertent complement activation.

CD59 is yet another widely distributed membrane inhibitory protein (61). It binds C8 in the C5b-8 complex and blocks the effective incorporation of C9. CD59 also binds C9 present in the evolving MAC and blocks the subsequent addition and polymerization of C9, thus stopping the complete formation of the transmembrane pore.

Another cell membrane protein inhibitor is complement C2 receptor inhibitor trispanning (62), which binds to C2 and blocks C2 cleavage to impair C3 convertase formation within the classical pathway. Though its role relative to other regulatory proteins is uncertain (63), sushi domain-containing protein 4 is another complement inhibitor. It contains SCR domains; is primarily brain restricted in expression; and partially inhibits complement deposition through the classical, lectin, and alternative complement pathways on the membrane in which it is expressed.

Once released in serum, the potent anaphylatoxins C5a and C3a undergo a rapid loss of activity that is primarily caused by C-terminal cleavage of Arg to make the desArg form (64). Serum carboxypeptidase performs this cleavage, which results in C3a and C5a derivatives with two to three orders of magnitude less cell-stimulating activity. Notably, impairment of this activity leads to a substantial increase in inflammation in the joints in a C5a-dependent manner (65).

Recent findings have added complexity to the relatively straightforward activities of the regulatory proteins described above. For example, deregulatory proteins from the complement Factor H–related (CFHR) protein family seem to counter the protective activity of Factor H in vivo. Described many years ago, CFHR proteins are part of the structurally related family that includes Factor H and are encoded by a series of genes that are physically adjacent to *CFH* on human chromosome 1. Notably, they each contain SCR domains with homology to subregions of Factor H (66, 67). Because many normal individuals lack expression either of CFHR1 and CFHR3 (*ΔCFHR3-1*) or of CFHR1 and CFHR4 (*ΔCFHR1-4*), these proteins were not thought to play critical roles. However, recently a large number of protective or risk associations with human diseases have been linked to deletions or variants of these genes (66, 67). Associated conditions include age-related macular degeneration (AMD), where *ΔCFHR3-1* is a highly penetrant protective factor (68), and aHUS, where *ΔCFHR3-1* is a disease risk factor and is associated with autoantibodies that interfere with Factor H regulatory function (69).

Recent studies, however, have clearly demonstrated that CFHR1, assembled into multimers either alone or in association with CFHR2 and CFHR5, primarily acts on surfaces to block Factor H binding, resulting in complement deregulation and enhanced local activation (70, 71). Self-association of these proteins to counter the binding of Factor H to surfaces is particularly relevant because Factor H forms tetramers in what is likely its most active form (58). Additionally, similar to Factor H, some CFHRs are captured by specific molecules on bacteria and other pathogens and block complement activation. Binding by Factor H may be a protective mechanism to complement activation on the pathogen by inappropriately blocking C3 convertases on the pathogen surface

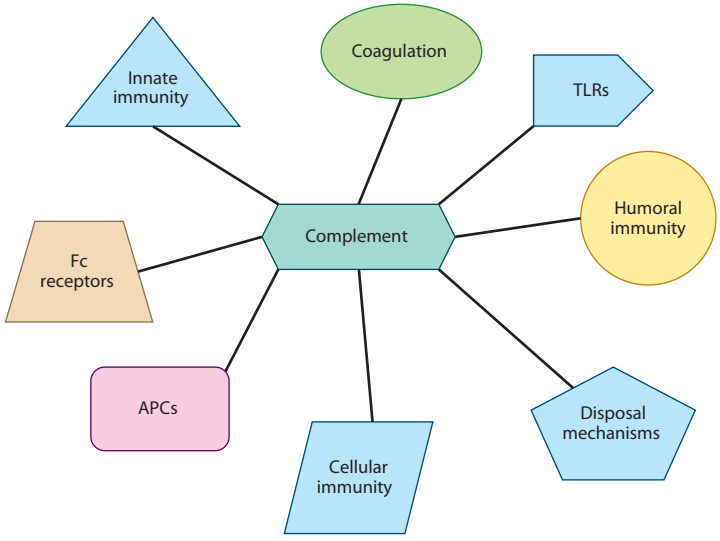

Figure 3

Subset of systems with which complement interacts. When activated, the complement system modulates and is influenced by many other biologically relevant pathways.

(reviewed in 67). Whether CFHR interactions provide an additional level of complexity exhibited by the immune system to counter this mechanism is under study.

CROSS TALK OF COMPLEMENT WITH OTHER SYSTEMS AND PATHWAYS

Coagulation

Having evolved in an evolutionarily early time frame as part of the innate immune and pathogen recognition/disposal mechanisms, the complement system is intertwined with other innate systems. It is also associated with more recently developed components including antigen-specific lymphocytes (1, 12). Several examples of such cross talk are present (**Figure 3**). One is the functional interaction between C5aR and IgG Fc receptors described above. The coagulation system is also built in a similar manner: It has multiple pathways, intrinsic and extrinsic activation mechanisms, and regulatory proteins and mechanisms (72). One of the functions of the coagulation system is to help physically wall off pathogens at sites of infection from the rest of the circulatory system (73). Accordingly, many serine proteases within the coagulation pathway directly cleave and activate complement proteins, and vice versa (72). Although it is not certain which of the many cross-talk mechanisms are biologically relevant, perhaps the most well established of these mechanisms in vivo is the ability of thrombin to cleave C5 and release biologically active C5a: During murine experimental pulmonary inflammatory injury induced by IgG, immune complexes can lead to C3-independent and thrombin-dependent injury (74).

The ability of MASP-1/3 activated by MBL to promote coagulation through thrombin cleavage provides another intriguing connection between the coagulation and complement pathways. In the setting of *Staphylococcus aureus* infection, the absence of such effects leads to the development of disseminated intravascular coagulation and liver injury (75). A related finding resulted from studies of samples from patients with thrombotic microangiopathies: Results showed that

microparticles derived from platelets and neutrophils during severe inflammation and thought to play roles in controlling coagulation are complement activating and are coated with C3 activation fragments (76). Finally, the clinical validity of this interaction is suggested by findings from plasma derived from patients with multiple injuries, where the early appearance of the complement activation product C5a, and its correlation with the coagulation biomarker designated thrombin–antithrombin complexes, was found (77).

Toll-Like Receptors

Toll-like receptors (TLRs) and complement are both components of the innate immune system and are activated by exposure to pathogens. Substantial cross talk occurs between the two systems during proinflammatory states that are characterized and driven by innate immune and T cell responses, especially during infection (78). If the regulatory capacity of complement is diminished, the effects of multiple TLR ligands (LPS, zymosan, CpG) are markedly enhanced by C5aR engagement, resulting in elevated plasma interleukin-6 (IL-6), tumor necrosis factor α (TNF-α), and IL-1β (79). The effects may be intracellular, reflecting the bidirectional effects on the mitogen-activated protein kinases Erk1/2 and Jnk (79), or they may be due to the physical association of anaphylatoxin receptors with TLRs that can also lead to subversion of the appropriate pathogen responses to some strains such as *Porphymonas gingivalis* (80).

The outcomes of combined TLR- and complement-dependent signals are highly dependent on the context in which they are experienced. Adaptive immune responses also vary depending on whether the antigen-presenting cells (APCs) are macrophages or dendritic cells and whether the APCs are activated or not. Each of these factors can strongly influence subsequent T cell phenotypes, which are also highly dependent on the type of APC and the context in which cytokine signals are delivered. Thus, concurrent TLR engagement, likely through macrophages, can promote proinflammatory outcomes through C5a and C3a receptors (81); additionally, the development of T helper 17 (Th17) cells is enhanced (82). Conversely, these same signals when acting on other targets can dampen the inflammatory response and alter the cytokine regulatory network that subsequently evolves. For example, TLR engagement in macrophages leads to the expression of IL-12 family cytokines, but coengagement of C5aR can markedly dampen this effect with a diminution in the development of Th17 cells and Th1 outcomes (83). However, these dampening effects manifest by complement activation are not found only with C5a or C3a signals: C1q receptor interactions can also downregulate human IL-12 production by macrophages and dendritic cells (84). Therefore, in a complex in vivo system whose output reflects highly integrated responses, it is difficult to predict a priori the phenotypic effects of an individual stimulus or combinations of stimuli without specific testing.

Adaptive Immunity

In addition to intersections with innate pathways, the complement system has evolved several mechanisms that strongly influence adaptive immunity. One of the first described was the ability of CR2 to act as a B cell coreceptor: Recognizing that C3d-bound antigens interact simultaneously with CR2 and the antigen-specific B cell receptor and that CR2 strongly associates with the signal-amplifying CD19 molecule, researchers determined that B cell activation would increase thousands-fold (45, 85). In addition to the direct effects of B lymphocyte surface activation, CR2 is also involved in antigen trafficking in the lymph nodes, which is necessary for subcapsular antigen capture and transfer into the B cell follicle to promote humoral immune responses (86).

With regard to cellular immunity involving T cells, C5a plays key roles. In asthma models, the timing of engagement with C5aR on dendritic cells leads to markedly different cytokine

elaboration as well as inflammatory and airway hyperresponsiveness (87). Further complexity has been added to the understanding of the effects of C5a and C3a by the reports that, when signals from C3aR and C5aR are not provided to $CD4^+$ T cells, signaling via the kinases PI(3)Kγ, Akt, and mTOR stops and activation of protein kinase A increases. When this occurs, autoinductive signaling by transforming growth factor-β1 (TGF-β1) is then allowed, and cells become induced $Foxp3^+$ regulatory T cells (Tregs). Additionally, TGF-β1 suppresses signaling through C3aR and C5aR by both impairing the production of C3a and C5a and upregulating C5L2, the alternative receptor for C5a. The lack of signals between C3aR and C5aR also results in decreased expression of costimulatory molecules and IL-6 and in increased production of IL-10 (88).

In addition to changes induced by C5a- and C3a-dependent mechanisms, engagement of complement regulatory proteins on T lymphocytes alters the subsequent activation and differentiation profiles. With regard to CD46, engagement in humans strongly influences the development of Tregs, which may be centrally important in the maintenance of peripheral tolerance and control of unwanted immune and autoimmune responses. Surprisingly, researchers first found that the engagement of CD46 on $CD4^+$ T cells led to the induction of adaptive Tregs, which acted through the release of IL-10 (as opposed to IFN-γ), production of granzyme B, and consumption of IL-2 (89). With regard to the mechanism(s) underlying this effect, more recent studies have shown that, in addition to complement activation fragments C3b and C4b, CD46 avidly binds Jagged1, a member of the Notch family. CD46 regulates the expression of Notch receptors and ligands during the process of T cell activation, and as expected, the absence of CD46 leads to markedly diminished induction of IFN-γ and IL-10 as well as to the absence of Th1 responses in vitro and in vivo (90).

INSIGHTS FROM GENETIC STUDIES OF COMPLEMENT

Although the study of genetic relationships to phenotypic outcomes has been quite informative in many fields, contemporary approaches have led to a remarkable number of insights into the roles of the complement system in human disease. Studies that have focused attention on the role of the alternative pathway in human disease have been particularly informative. In aHUS, inactivating mutations within the carboxy-terminal surface-binding domain of Factor H have been regularly found, suggesting a particularly important role for this molecule in controlling complement activation on and around the small arterioles in the kidney (13, 91, 92). In dense deposit disease, which also impacts the kidney, systemic fluid-phase activation occurs and mutations within the Factor H regulatory domain impair the ability of the molecule to control fluid-phase tick-over. This leads to a decrease in circulating C3 and an increase in C3 activation fragments in the glomerulus. Although researchers know from experimental murine models that the glomerulus requires the presence of Factor I to cleave C3b to the iC3b form, they do not know why the glomerulus is targeted in this disease. One proposal is that iC3b is preferentially bound or captured within the glomerulus, leading to damage (93). In C3 glomerulopathy (C3G), the study of mutations in CFHR proteins has been particularly informative. C3G is a renal disease characterized by C3 accumulation in the absence of the substantial concurrent presence of immunoglobulins (94). Recent studies have shown that mutations in C3 and copy number variants in CFHRs consisting of internal duplication and heterozygous hybrid proteins are closely associated with the presence of disease (71, 95, 96). In C3G, self-association through dimerization domains within certain CFHRs is important for their ability to interfere with Factor H surface binding that is essential for local complement control (70, 71). Additionally, in aHUS, genetic studies have demonstrated the presence of heterozygous mutations in the membrane cofactor protein (*CD46*) gene. Similarly, CD46 mutations have also been found in patients with fatal Stx-HUS and the HELLP (hemolysis, elevated liver enzymes,

and low platelets) syndrome. Such patients are deficient in their ability to control the complement alternative pathway on self-cell surfaces (97).

Other candidate gene approaches have also led to substantial insights. For example, with regard to the role of carboxypeptidase B, not only does inactivation of this gene in mice lead to substantial increases in C5a-dependent joint damage, but also patients with more severe rheumatoid arthritis (RA) as manifest by radiographic joint damage are more likely to have a polymorphic variant exhibiting a prolonged half-life (65). Additionally, analysis of complement regulatory proteins in a cohort of mothers who developed pre-eclampsia demonstrated a larger number of mutations in genes controlling the alternative pathway (98), consistent with studies in murine models where the complement pathway played roles in angiogenesis and fetal loss (99) and demonstrated features of pre-eclampsia when inappropriately activated (100).

Finally, there is the emerging concept of the "complotype" (101). This characteristic is defined as the relative rate of complement system in a given individual, which is determined by the functional characteristics of individual polymorphic variants of activation pathway and regulatory proteins. The complotype may play an important role in setting the relative risk of developing disease given other risk factors where a more active complement system may "tip the balance" toward injury.

THERAPEUTIC SUCCESSES IN TREATMENT OF RARE HUMAN COMPLEMENT-DEPENDENT DISORDERS

Several therapeutic approaches have yielded important insights into the role that the complement system plays in human disease. Most strikingly, complement modulators have been successfully developed for the treatment of rare human diseases that are caused by mutations.

Paroxysmal Nocturnal Hemoglobinuria

PNH is a human complement-mediated disease. It is characterized by the absence of DAF/CD55 and CD59 on clones of red blood cells, platelets, and leukocytes that is due to mutations in the gene *PIG-A* and by the subsequent inability for clones of cells to control complement activation (102). On red blood cells, the absence of DAF allows alternative pathway–initiated complement activation by C3 and C5 convertases; the absence of CD59 allows activated C5 to proceed to form the MAC, resulting in intravascular hemolysis (102, 103). Although the PIG-A mutation leads to a loss of all glycophosphatidylinositol-linked proteins from cell membranes, PNH is a complement-mediated disorder because treatment of patients with the anti-C5 monoclonal antibody eculizumab markedly abrogates hemolysis and its associated clinical sequelae (102, 103). Interestingly, because of continued apparent C3 convertase activity due to tick-over, covalently bound C3 fragments accumulate on PNH red blood cells (104), and in many patients, extravascular clearance of the cells through liver and spleen is promoted. As a result, the ability to achieve a normal cell count during treatment with eculizumab is abrogated.

Atypical Hemolytic Uremic Syndrome

aHUS is a rare but highly informative condition. It is associated with the presence of mutations in alternative pathway proteins, primarily Factor H (13, 105). Uncontrolled positive clinical trial results in pediatric aHUS have been reported using eculizumab, which has led to the approval of this drug for aHUS (106, 107).

Neuromyelitis Optica

As noted above, NMO is a rare disease associated with an IgG autoantibody to AQP4, which is thought to be a major driver of inflammation and astrocytic injury in the central nervous system (CNS). The findings of complement activation fragments in the lesions of patients as well as the results from animal studies have supported the idea that the pathway is pathogenic. Thus, seropositive patients with NMO with a history of ongoing disease despite optimal therapy were treated with eculizumab. Both the primary efficacy endpoint of the number of attacks and secondary endpoints were met (108).

Hereditary Angioedema

Hereditary angioedema is a disease caused by inactivating mutations in the classical pathway inhibitor C1-INH. It had historically been treated with purified protein obtained from plasma (53). Prophylactic therapy had relied on attenuated androgens or antifibrinolytic agents. More recently, though, newly approved replacement therapy for the treatment of hereditary angioedema attacks with recombinant C1-INH has been available, which has also opened up opportunities for prophylactic therapy in the appropriate patient subpopulation (109).

ADDITIONAL ROLES FOR COMPLEMENT IN COMMON HUMAN DISEASES: INSIGHTS FROM ANIMAL MODELS

Age-Related Macular Degeneration

Many studies in the past decade have shown that polymorphisms and rare variants of complement pathway genes whose products primarily promote activation of the alternative pathway are associated with a higher risk for the development of human AMD (110). AMD is characterized by a progressive loss of central vision that may be due to damage to the retinal pigmented epithelium and the subsequent death of photoreceptor cells in this region. The loss of photoreceptors appears to occur via two different mechanisms: One is primarily operative in the atrophic (dry) form of AMD, and the other in the neovascular or exudative (wet) form of AMD. Dry AMD is the more prevalent form, affecting up to 90% of all patients; however, the mechanisms by which photoreceptors degenerate is poorly understood. In wet AMD, vision loss appears to be due to choroidal neovascularization (CNV) and vascular leakage (110). Etiologically, oxidative stress (111) and complement activation (110, 112) may both contribute to the development of AMD.

Results from studies in several laboratories have suggested that complement activation in mouse CNV involves the alternative pathway (113, 114), especially given that eliminating the classical or the lectin pathway alone through gene-targeted deletion studies has no protective effect. In addition, the anaphylatoxins C3a and C5a (115), as well as the MAC (116), appear to contribute to disease severity. Notably, CNV experiments have suggested that vascular endothelial growth factor is an essential downstream molecule required for CNV and that the complement system controls the levels of this factor that are elaborated. With regard to a mechanistic linkage between oxidative stress and complement activation, in vitro treatment of retinal pigmented epithelial cells results in the development of complement-dependent injury (117). Recent clinical trials using complement antagonists in patients with dry AMD have also supported a potentially important role for complement.

Systemic Lupus Erythematosus

In murine models of human SLE, the complement system has been implicated in effector pathway–mediated target organ damage as well as in the effects on the development of autoimmunity in B and T cell lineages. With regard to effector mechanisms, treatment of MRL/*lpr* mice with a systemically active multiple pathway inhibitor ameliorates SLE (118). Notably, when mice lacking expression of Factor D are bred into this strain, renal lupus and other disease features are also diminished (119). Finally, use of targeted inhibitors of the alternative pathway in both (NZB × NZW)F1 and MRL/*lpr* mice produced a clinical benefit and led to multiple decreased measures of autoimmunity, including anti-double-stranded DNA antibodies, circulating immune complexes, glomerular C3, and proteinuria (120, 121).

Complement may also play a role in SLE and other autoimmune diseases via the interaction of CR2 with its C3d ligand. However, researchers do not yet know whether the need for CR2 expression is similar during the expansion of autoantibodies and foreign antigens. B lymphocytes and high-affinity autoantibodies play central roles in the immunopathogenesis of SLE (122, 123). Thus, in principle, given our current understanding of the immune basis for the development of pathogenic humoral autoimmune responses through subversion of normal tolerance checkpoints and the development of autoreactive rather than foreign specificities (124), the enhancing role found with foreign antigens may also be played by CR2 in its interactions with C3d-bound self-antigens. However, prior studies using gene-targeted $Cr2^{-/-}$ mice in murine models of SLE have demonstrated either an increase (125, 126) or no significant change (127) in autoimmunity and disease manifestations. Other recent studies have begun to shed new light on the problem: In both (NZB × NZW)F1 (121) and MRL/*lpr* (120) mice, the use of soluble CR2 as an experimental control for a complement inhibitor (itself capable of blocking C3d-CR2 interactions) demonstrated a substantial decrease in autoantibody development. Additional studies are under way.

Rheumatoid Arthritis

RA is an especially relevant disease process in which to study the pathogenic role of the complement system in both the effector phase and the development of autoimmunity. RA is an important human autoimmune disease, exhibiting the second-highest prevalence of any autoimmune disease (128, 129). The pathogenesis of RA can be divided into three distinct phases: initiation, perpetuation, and chronic inflammation. Innate immune mechanisms involving the complement system are likely to be involved in all three stages (130, 131). RA also extensively involves both the cartilage and synovium. With regard to the cartilage surface, studies have demonstrated the presence of IgG-containing immune complexes as well as complement C3 activation fragments in >90% of patient samples (132). The synovium is also a site of extensive complement deposition and synthesis in patients and animal models (133, 134).

With regard to effector mechanisms, studies in murine models have demonstrated that complement, especially the alternative pathway, is key to initiation and amplification. Complement is also central to driving these mechanisms (135, 136), which appear to encompass C5aR, C3aR, and the MAC (137). In addition, cartilage-derived proteins in RA exhibit both complement activating and regulatory functions (138). A model of initiation and propagation of joint inflammation and damage has been developed to illustrate this and other disease states within an experimental setting (**Figure 4**). With regard to adaptive immunity, the interaction between CR2 and C3d also plays an important role in RA animal models, where deficiency of the receptor leads to a decrease in clinical disease activity and tissue damage as well as in pathogenic anticollagen and other disease-specific autoantibodies (139).

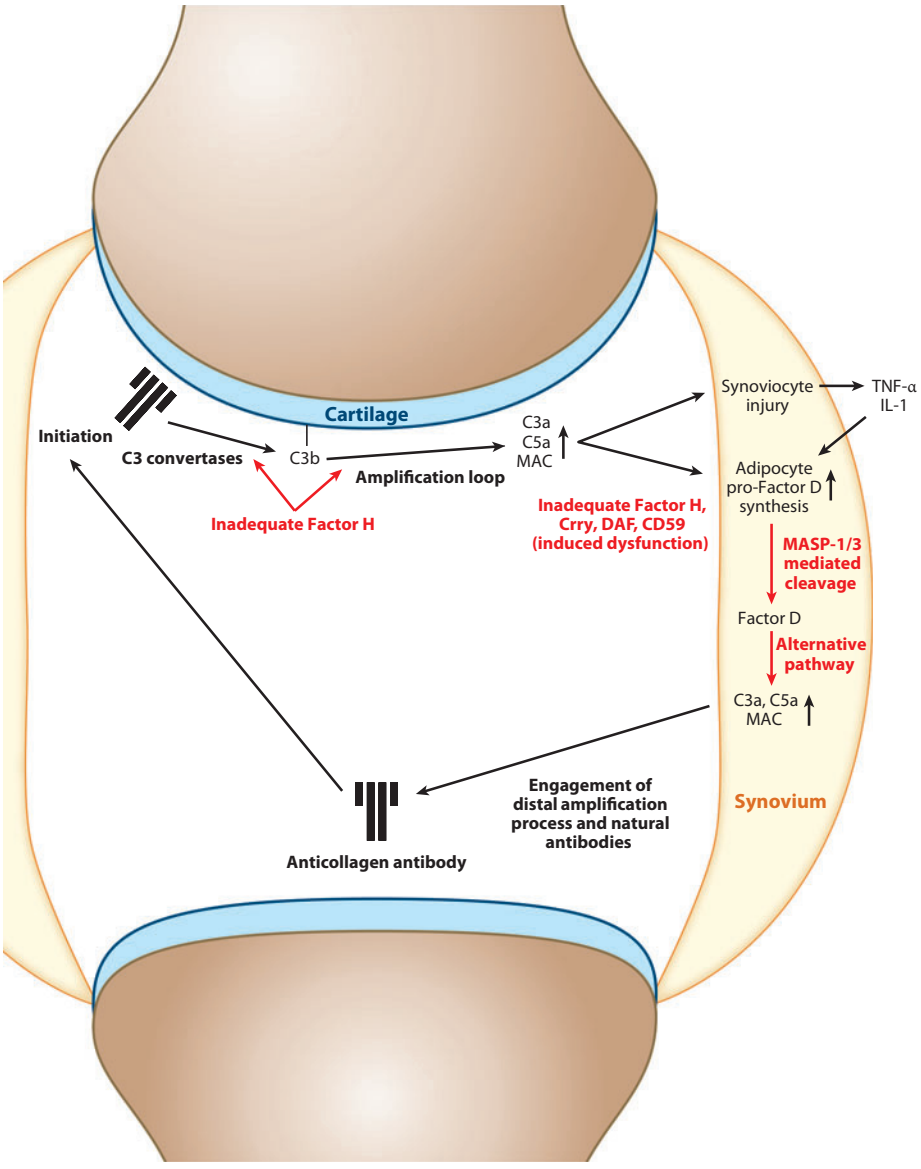

Figure 4

Model of complement activation, effector, and regulatory activities in compartments in the arthritic joint. When anticollagen antibodies enter the joint, they activate the complement classical, lectin, and alternative pathways and then lead to injury through the amplification loop. In the process, following infusion and the formation of immune complexes on the cartilage surface, Factor H activity is insufficient to control complement activation, and neither Factor H nor the membrane regulatory proteins MCP, DAF, and CD59 are able to protect synoviocytes from subsequent damage and development of a proinflammatory phenotype. Adipocytes also likely play a proinflammatory role by locally producing pro-Factor D that is cleaved by MASP-1/3 to the active Factor D molecule, thereby priming activation of the alternative pathway amplification loop. In parallel with these events, the generation of effector molecules (C5a, C3a, and the MAC) leads to the engagement of multiple downstream injury mechanisms. [Abbreviations: Crry, complement receptor related protein-y; DAF, decay-accelerating factor; MAC, membrane attack complex; MCP, membrane cofactor protein (also known as CD46).]

Neurodegenerative Disorders

The complement system exhibits a complex relationship within CNS development and neurodegenerative disorders, the latter exemplified in Alzheimer's disease. During development, C1q is expressed by postnatal neurons in response to signals from immature astrocytes and is localized in synapses in both the CNS and retina. This is biologically important because mice deficient in complement protein C1q, or the downstream complement protein C3, demonstrate substantial defects in CNS synapse elimination, suggesting that inappropriate connections are eliminated in a complement-dependent manner. Although C1q is normally downregulated in the adult CNS, it is inappropriately upregulated and relocalized to the synapse in a mouse model of glaucoma, thus potentially playing a role in inappropriate neuronal elimination (140).

Inappropriate regulation may extend to Alzheimer's disease, where complement activation products have long been known to accumulate in the plaques and likely to promote the development of local inflammation that leads to cognitive dysfunction. However, some of the complement components, as noted above, contribute to neuroprotective pathways, and the relative balance of complement activation likely plays a role in modulating neuronal function. Consistent with these concepts, when generation of the complement activation product C5a, which recruits and activates microglia and astrocytes in vitro by activating a G protein–coupled cell-surface C5aR, was inhibited, substantial reduction of pathological markers was noted in a mouse model of Alzheimer's disease (141).

Type 1 Diabetes Mellitus

Although type 1 diabetes mellitus has been considered the prototypic T cell autoimmune disease, an intriguing link to complement was recently found. In the nonobese diabetic mouse strain, insulitis develops, followed by hyperglycemia and β cell destruction. Researchers found expression of the complement receptor CRIg marked a subset of macrophages associated with diabetes resistance. Strikingly, administration of a fusion of CRIg and the Fc portion of immunoglobulin led to a substantially lower level of diabetes incidence (142).

Sepsis and Other Acute Inflammatory Disorders

Severe sepsis is an acute condition that leads to activation of both the complement and coagulation cascades that have been proposed to play central roles in multiple organ failure and severe complications; however, the pathophysiology is incompletely understood. In a baboon model of sepsis-induced multiple organ failure, administering the complement C3 convertase inhibitor compstatin after *Escherichia coli* challenge led to substantial clinical benefit as well as inhibited sepsis-induced blood and tissue biomarkers of complement activation, reduced leucopenia and thrombocytopenia, lowered accumulation of macrophages and platelets in organs, decreased complement activation-associated idiopathic response by downregulating tissue factor and PAI-1, diminished global blood coagulation markers (fibrinogen, fibrin-degradation products, APTT), and preservation of endothelial anticoagulant function (143). Thus, complement activation may play an especially important role in tissue injury during sepsis.

Cancer

There are many roles proposed for complement in cancer and cancer therapies. For example, because elevated levels of complement regulatory molecules expressed on tumor cells provide an

evasion mechanism against monoclonal antibody therapy, they may also modulate the development of an acquired antitumor immune response. Investigators conducted a study and found that the enhancement of complement activation by therapeutic monoclonal antibodies also enhances target cell killing (144). In a related approach, a fusion protein, CR2-Fc, containing an iC3b/C3dg recognition domain through CR2 and complement activating capacity through the Fc domain amplified therapeutic antibody-dependent complement activation and tumor cell lysis both in vitro and in vivo (145).

In other models, complement activation products have also been shown to promote tumor growth. Specifically, complement C5a in a tumor microenvironment substantially enhances tumor growth by suppressing the antitumor $CD8^+$ T cell response. This process was associated with the recruitment of myeloid-derived suppressor cells and the enhancement of their antitumor T cell suppressive abilities (8, 146).

Transplantation-Related Cellular Regeneration

Though not widely appreciated, modulation of cellular proliferation is another process in which complement plays an important role. One of the best examples of this phenomenon is in liver resection and transplantation. Major liver resection and small-for-size liver transplants pose challenges as a result of the increased susceptibility of the remnant/graft to ischemia reperfusion injury and impaired regeneration. The role of complement in this process has been examined in several animal models. One study showed that low-dose complement therapeutic treatment was protective and increased hepatic proliferative responses relative to control mice and that C3a-degradation product acylation-stimulating protein as well as C5a plays important roles in controlling the balance between inflammation/injury and regeneration (10, 147). Furthermore, IL-4 is also important in liver regeneration: Cytokine production by NKT cells that accumulate in regenerating livers after partial hepatectomy is essential for the protective process because they regulate the local activation of complement after liver resection.

CONTEMPORARY CHALLENGES AND OPPORTUNITIES

Need for Systems Biology Approaches

The complement system is complex: It includes multiple activation and regulatory pathways, receptors for many activation fragments, context-dependent effects on adaptive immune responses, and strong in vivo influences of polymorphic variants on outcomes. Thus, it is a challenge to predict in vivo outcomes of therapeutic manipulation. In this light, studies of the complement system will likely greatly benefit from the application of advanced genotyping and systems biology approaches (12). Because risk alleles are incompletely penetrant (66), the contributions of additional genes and as yet unknown environmental stimuli will also need to be defined.

Improved and More Informative Biomarkers for Complement-Dependent Disorders

Within the complement system, aberrations in the levels of components have traditionally been used to reflect the presumed activation of individual complement pathways during many pathological conditions such as autoimmune diseases, infections, cancer, allogeneic and xenogeneic transplantation, and inflammation. By analyzing groups of patients and controls in many disease states, researchers can easily detect elevation. However, it has not been possible to make

predictions for individual patients because many of the assays used lack sufficient sensitivity and specificity. In addition, in patients very little is known about the relationships between the levels of therapeutic complement inhibitors and their effects on currently available biomarkers (148).

The use of directed sequencing in candidate gene analyses may provide a molecular "window" into the pathogenesis of aHUS and other related renal diseases (66). Additional linked genetic/genomic/epidemiologic approaches should continue to guide our understanding of the class of diseases with similar alterations in complement and coagulation system genes. Doing so may lead to insights similar to those found for autoimmune diseases that share identical loci with major immune-mediated diseases (149).

Finally, as noted above, the use of current biomarkers to assess complement activation has substantial limitations, especially at specific tissue sites. As such, new imaging strategies may prove very helpful. In one approach, researchers developed murine monoclonal antibodies cross-reactive against human and mouse C3d, which is the final C3-degradation fragment generated during complement activation. The most informative antibodies bind to tissue-bound C3 activation fragments when injected systemically. Using optical imaging to develop a model of CNV, researchers also identified C3 fragment deposition in live mice with induced retinal lesions. Thus, it may soon be possible to detect and monitor complement activation-associated tissue in a manner that would directly inform clinical decision making (150).

Targeted Therapeutics

To date, approved complement therapeutic strategies have largely followed traditional paths involving replacement factors (C1-INH) and inhibitory monoclonal antibodies (eculizumab). Similar approaches are being applied to many other targets within the system (18), while additional approaches are also being developed. One approach is based on the concept that directing (targeting) therapeutics to sites of complement activation, using a number of strategies and molecules that specifically bind to these activation fragments, should, in principle, provide substantial benefits over systemic treatment approaches (151).

CONCLUSION

The complement system is a complex pathway that exhibits multiple mechanisms of activation, regulation, and effector functions. Complement activation proteins interact with many other innate and adaptive immune processes as well as "nonimmune" pathways to provide an integrated response to infection and other stimuli. Complement inhibitors have demonstrated efficacy in several diseases associated with genetically driven dysregulation. Finally, human and animal model studies have strongly suggested that inappropriate activation of this pathway plays a causal role in damaging tissues in many other autoimmune and inflammatory diseases.

SUMMARY POINTS

1. The complement system plays major roles in modulating both innate and adaptive immunity.
2. A major functional linkage exists between complement and other systems, including coagulation, adaptive immunity, Fc receptors, TLRs, and noninflammatory disposal mechanisms.

3. Inappropriate complement activation is pathogenic in human diseases driven by genetic mutations, "activating" complement pathway polymorphisms and pathogenic autoantibodies that drive complement activation.

DISCLOSURE STATEMENT

V.M.H. has multiple submitted and issued patents in the area of complement therapeutics and diagnostics, has received royalty payments for licensed patents, and has previously worked as a consultant for complement therapeutic companies.

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