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The Evolving Landscape for Complement Therapeutics in Rheumatic and Autoimmune Diseases

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Introduction

The complement system is a major component of innate immunity [1] and plays central roles in protective immune processes including pathogen clearance, recognition of foreign antigens, amplification of humoral and cellular immunity, removal of apoptotic cells and debris, and promotion of organogenesis and regeneration of certain tissues such as the liver following transplantation [2, 3]. In contrast, inappropriate complement activation directed against self-tissues underlies the pathogenesis of a number of human inflammatory and autoimmune diseases. The successful development of complement therapeutics, as well as the presence of a substantial pipeline of drugs under development [4], has advanced opportunities for application to rheumatic and inflammatory diseases. Herein we will briefly review the complement system and then focus on two topics. The first is the emerging understanding of the pathogenic roles of complement activation in rheumatic and closely related diseases. The second is an update regarding contemporary complement pathway testing methodologies to answer questions in both clinical settings and therapeutic development programs.

Initiation, Amplification, Regulation and Effector Pathways in the Complement System

Activation

The complement system comprises three activation pathways, classical (CP), alternative (AP) and lectin (LP) (Figure 1). The CP is initiated through antibody dependent and independent means, which act as the “targeting” mechanism to direct the powerful effector arms of complement to specific sites [5]. IgM and IgG antibodies activate the CP, while IgA

can activate the AP and IgE is not an effective complement activating isotype. Following antigen recognition and binding by C1q of the tripartite C1 complex, autocatalytic activation of C1r and transactivation of C1s results in the latter molecule sequentially cleaving C4 and C2 into C4b and C2a, respectively. These two proteins associate non-covalently as the CP C3 convertase C4b2a. Antibody independent CP activators, which may be particularly important when products of tissue damage are elaborated, include the pentraxins C-reactive protein (CRP) and PMX as well as beta amyloid fibrils, serum amyloid P and mitochondrial proteins; each of these molecules directly interact with C1q and activate C1r/C1s. C1q also exhibits additional functions involving the clearance of apoptotic debris and regulation of pruning of synapses [6].

The LP is initiated using different target recognition molecules designated collectins, a family which includes mannose-binding lectin (MBL), Ficolins 1–3 and CL-11 and is involved in protective clearance through the recognition of invariant features of foreign organisms [7]. The LP is also engaged during tissue injury through the direct recognition of proteins such as cytokeratin-1 and other less well characterized ligands, as well as IgM/IgG antibodies containing agalactosyl (G0) carbohydrates [8]. As in the CP, connectivity to downstream generation of C3 and C5 convertases occurs through the engagement and conformation-dependent elaboration of proteases, here MBL-Associated Serum Proteases (MASP)-1, MASP-2 and MASP-3 that cleave and activate C4, C2 and possibly C3 directly.

The AP, in contrast, is unique in being initiated through spontaneous conformational changes of C3 in a process designated “tick-over”, which results in binding of Factor B and cleavage by Factor D, stabilization (and in some settings directed focusing) through Properdin binding, and the ultimate formation of the C3 initiation convertase C3(H₂O)BbP [9]. This process is accelerated through the binding of C3 to surfaces including gas bubbles, platelet surfaces, biomaterials and microparticles as well as a convertase stabilizing autoantibody designated C3 nephritic factor and some immunoglobulin light chains. Importantly, the same components also serve as an “amplification loop” following C3b fixation to targets through either of the three pathways [10], which is an essential mechanism for complement-dependent injury *in vivo* [11].

During the processes of C3 activation by its convertases, the thioester bond in C3 is broken, allowing the formation of covalent ester or amide linkages from C3b to neighboring molecules [12]. The subsequent sequential cleavage of C3b to iC3b/C3dg/C3d by Factor I and cofactors provides the capacity to interact with specific high affinity/avidity C3 receptors [13]. The formation of C3b is followed by the formation of the multi-protein CP and AP C5 convertases, C5 cleavage to C5b and assembly of the pore-like membrane attack complex (MAC). Also generated through the convertase activation processes are the soluble anaphylatoxins C3a and C5a, which have multiple pro-inflammatory and complex immunoregulatory roles [14].

Receptors

High affinity complement receptors are engaged by proteolytic cleavage fragments generated during the activation process. Receptors include complement receptor type 1 (CR1, CD35), a widely distributed molecule that acts as the major receptor on erythrocytes

for binding and processing circulating immune complexes containing C3b and/or C4b, acts on B cells to modulate responses to antigens [15], and functions on neutrophils and macrophages as a phagocytosis-promoting receptor [16]. A second receptor, which binds the iC3b and C3d fragments, is CR2/CD21, a receptor expressed on B cells, epithelial cells, follicular dendritic cells (FDC), thymocytes and a subset of peripheral T cells. CR2 promotes B cell activation, traps and retains immune complexes on follicular dendritic cells (FDC) within lymphoid tissues and serves as a modulator of B cell self-reactivity [15, 17]. CR3 and CR4 are integrins that bind iC3b, are expressed on phagocytes and FDCs and exhibit immunoregulatory roles [2]. Another receptor is Complement Receptor of the Immunoglobulin superfamily (CRIg), which is expressed on Kupffer cells and mediates clearance of targets from the circulation. The anaphylatoxic peptides, C5a and C3a, exhibit multiple pro-inflammatory properties and are each recognized by receptors, C5aR/CD88 and C3aR, which are members of the rhodopsin family [18]. C5a is a particularly important molecule, demonstrating multiple pro-inflammatory properties, including leukocyte chemotaxis, aggregation of neutrophils and platelets, release of mast cell mediators, generation of leukotrienes, cytokines and reactive oxygen metabolites, and activation-modulating cross talk with IgG Fc receptors [19]. Similarly, C3a plays key roles in inflammatory disorders [20].

Regulators

Several mechanisms are utilized to restrain complement activation. For example, the CP and LP are blocked by C1-inhibitor (C1-INH) [21], which serves as a trap for C1r/C1s and activated MASP-1/MASP-2. C1-INH also inactivates the proteases kallikrein, factor XIa, XIIa, and plasmin of the contact and clotting systems. In addition, membrane and circulating proteins accelerate the decay and inactivation of the C3 and C5 convertases [22]. These include Factor I, which cleaves and inactivates/processes C3b and C4b at specific sites when they are either free in the fluid phase or target-bound. Factor H, a soluble inhibitor, is a decay-accelerator of the AP and also serves as a cofactor for Factor I mediated cleavage of C3b. With regard to the CP, C4b-binding protein (C4-bp) is a fluid phase protein that exhibits similar mechanisms as Factor H.

Proteins that block complement activation on the cell membrane include DAF (decay-accelerating factor, CD55), MCP (membrane cofactor protein, CD46) and MIRL (membrane inhibitor of reactive lysis, CD59) [23]. DAF binds C3b or C4b and increases the spontaneous decay of both the CP and AP C3 complexes. Membrane cofactor protein (MCP/CD46) serves as a cofactor for the cleavage of C3b and C4b into their inactive forms. CD59 binds C8 to block the effective incorporation of C9 and also blocks C9 polymerization, which is required for pore formation. Finally, C5a and C3a undergo a rapid loss of activity mediated by C-terminal cleavage of Arg by carboxypeptidase [24].

Another type of regulatory activity is manifest by members the Complement Factor H Related (CFHR) family, for which many associations with human diseases have been found [25]. These conditions include age-related macular degeneration (AMD), atypical hemolytic uremic syndrome (aHUS), IgA nephropathy and systemic lupus erythematosus (SLE). In

this regard, some CFHR members appear to act as inhibitors of Factor H by blocking its binding to target surfaces, resulting in enhanced local complement activation.

Overview of Complement Therapeutic Approaches

There are two currently approved complement therapeutics [26]. The first targets C5 and is represented by Eculizumab [27], while the second encompasses a number of molecular versions of C1-INH [28]. Eculizumab is approved for the treatment of paroxysmal nocturnal hemoglobinuria (PNH) and aHUS [29], while C1-INH preparations are approved for hereditary angioedema (HAE). In addition, therapeutics are under development which are directed to molecules including the C3 and C5 convertases, C3 itself, C1s, MASP-2, MASP-3, Properdin, Factor D, Factor B, C5, C5a, C3a and C6 as a means to disrupt the MAC (Figure 1, Table 1). In addition to the soluble proteins, inhibitors of receptors are being evaluated. Therapeutic modalities include monoclonal antibodies, recombinant forms of natural inhibitors, small molecules with various backbones and structures, RNA-based drugs, other recombinant proteins, modular chimeric proteins and gene therapy. In addition, tissue- or cell-targeted therapeutics are under development, where the goal is to direct inhibitors more directly to sites of complement activation [30].

Disease indications that are currently under evaluation include myasthenia gravis and neuromyelitis optica. Additional focus is on local delivery, especially into the eye for age-related macular degeneration (AMD), as well as C3 glomerulopathy, a disease characterized by associations with AP-promoting activating mutations and autoantibodies [22]. Challenges in the development of complement inhibitors include the high levels of circulating proteins, which are much higher than cytokines. Additional challenges include the relatively rapid turnover, the acute phase response-mediated increases in circulating factor levels, and the potent biologic activity of locally synthesized factors [26]. The risks inherent in the use of systemic complement inhibitors, especially chronically, likely mirror the phenotypes seen in patients with homozygous complement deficiencies [31] and include disruption of immune complex processing, bacterial infection and potentially the impairment of cellular regeneration and repair cycles. Practical experience in the area of infection risk mitigation has been gained through the clinical use of Eculizumab and the means by which infection with *Neisseria* has been partially mitigated through chronic antibiotic use and close monitoring [27].

Use of Complement Therapeutics in Genetically Defined and Related Diseases in the Differential Diagnosis with Rheumatic Diseases

There is a wealth of experimental data in animal models pointing to the importance of the complement system in causing damage [2]. However, the most important insights have been gained through human therapeutic trials for rare human diseases caused by mutations that directly affect the complement system.

Paroxysmal Nocturnal Hemoglobinuria

PNH was the first disease in which the major complement-dependent aspects (red blood cell lysis, platelet/endothelial cell activation and clotting) were able to be therapeutically blocked [27]. PNH is caused by somatic mutations in the gene *PIG-A* that result in the absence of DAF/CD55 and CD59 on clonal populations and the subsequent inability of these lineages, especially RBCs, to control spontaneous AP-mediated complement activation. Treatment of patients with Eculizumab markedly abrogates hemolysis and other associated clinical sequelae, inclusive of thrombotic events that were previously the primary cause of death [32].

Antibody-Mediated Hemolytic Syndromes

The complement system has long been thought to play important roles in these syndromes, especially cold agglutinin disease (CAD) where activation of complement leads not primarily to direct lysis but rather the clearance of IgM-coated RBCs in the liver through a process designated extravascular hemolysis. Recent therapeutic development in this area has included the use of a monoclonal antibody inhibitor of C1s, which is demonstrating promise in studies in patients with CAD [33]. The approach of blocking complement activation through the CP would also be appropriate to test in patients who demonstrate autoimmune hemolytic anemia.

Hereditary Angioedema (HAE)

This is a disease associated with heterozygous mutations in the gene encoding inhibitor C1-INH, which has been treated with the purified protein obtained from plasma as well as anti-fibrinolytic agents and attenuated androgens [34]. Replacement therapy with recombinant C1-INH for HAE attacks has been approved, which has also opened up potential opportunities for its use in other diseases.

Atypical Hemolytic Uremic Syndrome

aHUS is a multi-organ disease caused by complement-mediated inflammation and thrombosis in the microvasculature. Patients typically present with thrombocytopenia, hemolytic anemia and renal failure. Most cases of HUS (~90%) are caused by enteric infections with bacteria that produce Shiga-like toxin (Stx), and the term “atypical” is used to describe those patients who develop the disease in the absence of Stx-producing bacteria. The majority of aHUS patients have genetic or acquired defects in their ability to control AP activation [35]. Disease-associated mutations have been identified in the genes for factor H, MCP, Factor I, C3, factor B, and thrombomodulin. Mutations in the complement regulatory proteins impair complement regulation by the affected proteins, whereas mutations in the *C3* and *CFB* genes are gain-of-function mutations. Some aHUS patients have inhibitory autoantibodies which impair the ability of Factor H to control AP activation [36]. Two Phase II studies of Eculizumab in aHUS [37] led to significant clinical improvement, and additional studies also confirmed that Eculizumab is an effective treatment for this disease [38].

Uveitis, Age-Related Macular Degeneration (AMD) and Other Ophthalmologic Disorders

Based on an extensive literature in experimental disease models [39], the complement system has been considered to play a major role in the pathogenesis of autoimmune uveitis. Additional impetus for studies in ophthalmologic disorders has been provided by the findings that informative polymorphisms and rare variants of complement genes are associated with a heightened risk for the development of AMD [40]. Formal proof of the role of the AP in AMD should emerge shortly from late stage studies of the use of a monoclonal antibody inhibitor of the AP protease Factor D.

Rheumatic Diseases That Are Likely in Part Complement Dependent

Rheumatoid Arthritis (RA)

RA affects 0.8–1.0% of the population and is associated with substantial personal and societal costs [41]. 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 each [42]. Studies have demonstrated the presence of IgG-containing immune complexes as well as complement C3 activation fragments in >90% of patient cartilage samples [43], as well as extensive synovial complement deposition and synthesis [44]. Notably, an unbiased analysis of the epigenetics of RA fibroblast-like synoviocytes revealed that complement system was among the top six pathways identified as expanded in RA samples [45]. Studies in murine models have revealed that complement is key to initiation, amplification and driving effector mechanisms [46]. In addition, cartilage-derived proteins in RA exhibit both complement activating and complement regulatory functions [47]. Experimental models have identified key roles for the AP and LP initiation and amplification mechanisms, chemotactic peptides and their receptors, local complement synthesis and release and dysregulation mechanisms on cartilage and cells. A mechanism-based model is shown in Figure 2. With regard to adaptive immunity in RA, the CR2-C3d interaction plays an important role in murine models, where inhibition of the receptor leads to a decrease in clinical disease activity, tissue damage, and development of pathogenic anti-collagen and other disease-specific autoantibodies [48]. Enthusiasm with regard to the use of complement inhibitors in RA has waned with a negative result using a C5aR antagonist in patients [49], although the increasing understanding of the immune complex mechanisms underlying the initial joint inflammation suggest that earlier treatment may be more effective [50].

Systemic lupus erythematosus (SLE)

The role of complement in SLE is paradoxical insofar as the complement system helps to prevent autoimmunity, yet it is also an important mediator of inflammatory tissue injury in SLE. Individuals with deficiencies of C1, C2, and C4 are all at increased risk of the disease [51]. The complement system is believed to prevent autoimmunity by several mechanisms. Intracellular antigens are released when cells die, and opsonization of the antigens by complement facilitates their rapid phagocytosis [52], which may reduce the likelihood of an autoimmune response to the exposed self-antigens. Complement activation may also improve B and T cell tolerance to self-antigens [53]. Once autoimmunity develops, circulating immune complexes deposit in tissues or form in situ when antibodies bind to

tissue antigens. Deposited immune complexes activate the complement system, with several pro-inflammatory and cytotoxic effects (Figure 3). It stands to reason that this inflammatory process causes more severe injury in those patients with reduced ability to control complement activation, and genetic studies suggest that this is the case [54]. Opsonization of injured cells with complement proteins may also amplify production of type I interferons [55]. Ideally, treatment regimens for patients with lupus would rapidly block the inflammatory effects of immune complexes within tissues and also modulate the adaptive immune responses that underlie autoantibody generation. A rational approach might therefore be to combine a complement inhibitor with drugs that target the adaptive immune response. This combination could prevent irreversible injury by immune-complexes already deposited in tissues while awaiting the adaptive immune effects. Notably, Eculizumab has been successfully used off label in the setting of severe lupus nephritis, even in the absence of TMA lesions [56], although no large series or trials have been conducted.

The optimal complement inhibitory drug in SLE would block the pathogenic effects of complement without impairing its role in protecting from autoimmunity. This outcome could be achieved by selectively blocking the AP and by using drugs that are targeted to sites of tissue injury [30].

Vasculitis

Several autoimmune diseases can present with small vessel vasculitis and glomerulonephritis. Immunofluorescence microscopy of tissue biopsies in these diseases reveals prominent immunoglobulin and complement deposits. Tissue biopsies from patients with anti-neutrophil cytoplasm antibodies (ANCA) associated vasculitis, in contrast, display only small quantities of immune deposits (“pauci-immune”). Nevertheless, a growing body of experimental and clinical observations indicates that complement activation is critical to the development of ANCA-mediated disease.

A role for complement was first shown in a murine model of ANCA vasculitis, where complement depletion with cobra venom factor, Factor B deficiency and C5 deficiency were each protective [57]. In a subsequent study, the authors found that ANCA cause neutrophil activation in vitro, and factors released from the neutrophils – including reactive oxygen species - caused generation of C5a when mixed with serum [58]. C5a, in turn, increased expression of ANCA antigens by neutrophils and primed them to respond to ANCA. ANCA thereby induce a feedback loop in neutrophils, and C5a is an essential intermediary. Using an in vivo model of ANCA vasculitis, the authors found that mice with C5a receptor-deficient bone marrow developed significantly milder disease than mice with wild-type bone marrow. Although C3 deposits are less prominent in glomeruli of patients with ANCA vasculitis than in immune-complex diseases, they are detected in more than 40% of patients and correlate with poor outcomes [59]. In addition, complement activation fragments (Bb, C3a, C5a, sC5b-9) are also elevated in plasma and urine [60] of patients with active disease, indicating that the AP is activated. Based on the efficacy of CCX168 in the murine model and the evidence of complement activation in human patients, a phase II trial of CCX168 was conducted [61]. This study randomized patients to three different treatments: standard therapy (corticosteroids + cyclophosphamide), standard therapy with reduced prednisone

dosing + CCX168, or CCX168 used in lieu of corticosteroids. The rate of renal remission and the reduction in the BVAS scores was greater in the groups that received CCX168, and additional studies are underway that will compare the efficacy of CCX168 with prednisone at inducing remission when the drugs are used in combination with either cyclophosphamide or rituximab.

Anti-Phospholipid Antibody Syndrome (APS) and Catastrophic Anti-Phospholipid Syndrome (CAPS)

The APS is a clinical syndrome diagnosed detection of these antibodies in patients who have developed thrombotic events or recurrent fetal loss. CAPS refers to a syndrome in which multiple organs are affected, and it can lead to multi-organ failure or death. An important role for the complement system in APS was identified by several studies that used antibodies from human patients to cause pregnancy loss in mice [62]. These studies revealed critical involvement of the CP. LP and C5a in the pathogenesis of APS. C5a and the MAC cause endothelial cell activation, adhesion molecule expression, cytokine release, and the release of pro-thrombotic molecules such as tissue factor and plasminogen activator inhibitor-1 (Figure 4). C5a also induces neutrophils to release tissue factor and monocytes to release soluble vascular endothelial growth factor-1 (sVEGF-1) [63]. Thrombin and fibrinogen catalyze complement activation, so there is probably microvascular cross-talk between the complement and coagulation cascades.

There is clinical evidence of complement activation in patients with APS, where C3 and C4 levels are lower in patients compared to control patients, and levels of C3a and C4a are elevated [64]. There are now several case reports of patients with refractory disease successfully treated with Eculizumab. Recurrence of APS is common after kidney transplantation, particularly in patients with CAPS, and case series have been published of high risk CAPS patients who were prophylactically treated with Eculizumab to prevent disease recurrence in renal transplants [65].

Neuromyelitis Optica (NMO)

NMO is associated with and likely caused by the actions of complement activating IgG autoantibodies to aquaporin 4, which act as major drivers in experimental injury models. In support of a role for complement in this disorder, autoantibody positive patients have been treated successfully with Eculizumab, as manifest by decreasing the number of attacks as well as achieving secondary endpoints [66].

Complement System Diagnostic Approaches: Protein, Functional and Genetic

The recognition of the role of complement in human diseases and interest in complement therapeutics have increased the need for robust complement analysis. The currently utilized complement diagnostic testing can be divided into four general categories: component protein levels, pathway function assays, activation marker levels in both the circulation and deposited on cells, and analysis of complement genetics. Tests of the levels of the complement components, particularly C3 and C4, are the most common complement tests

and have proved useful for following a number of rheumatic disorders. However, these assays have shortcomings that should be considered, especially where readily available tests for the levels of complement components does not identify whether the C3, for example, is intact or cleaved. In addition, many of the complement components are acute phase reactants, so measured levels balance disease-related consumption against increased production [67].

The second and most historic category of assays is the functional assay, particularly of CH50. The interest in functional testing has increased with the advent of complement-inhibiting therapeutics, and new methodologies have been designed to simplify functional testing. For instance, historic hemolytic assays are now joined by assays that utilize engineered lysosomes whose disruption is measured by ELISA style assays that are more amenable to the routine immunology laboratory. All functional assays represent appropriate screening approaches for the whole complement pathway, from activation through the terminal complex. However, if a functional assay is going to be used to assess a patient on complement therapeutics, it is important to note that standard assays require substantial dilution of the patient serum, which may artificially dilute the drug that is being tested and yield a result that does not parallel the *in vivo* inhibition. The AP hemolytic assay, AH50, utilizes lower dilutions and could, therefore, be the better choice for following patients on a complement inhibitor.

The final class of protein level analysis of complement testing is the measurement of complement activation markers. These include the split products that are produced during the cleavage cascade of complement activation, as well as testing the convertases and complexes also produced upon complement activation, which therefore have the potential to serve as markers of ongoing or uncontrolled activation. The markers may be measured in circulation by standard immunologic testing or as deposited on cells by flow cytometry. These tests may reveal the specific pathway involved or inhibited and the extent of complement modulation that is occurring. This has led to particular interest in the terminal complement complex sC5b-9 use for C5 inhibitors. What has reduced adoption of activation marker testing revolves around availability as well as the requirement for rapid processing and freezing of the specimens [68]. Specifically, as the complement system is very prone to *ex vivo* activation, activation markers can increase (or functions decrease) substantially if not handled properly and frozen quickly at -80°C [69].

Interest in complement genetic testing has also increased. A number of novel mutations have been associated with aHUS as well as with C3G and with overlapping patterns of AMD (see Table 1) [70, 71]. Because certain mutations in Factor H, in particular, are clearly mechanistically connected to pathogenesis, complement genetic testing can aid in diagnosis and prognosis. In addition, pairing complement genetic testing with phenotypic testing can be informative in determining the biological outcome of novel mutations.

Finally, additional methods are being evaluated that will utilize imaging of local complement activation *in vivo* and will provide insights into the local levels of fixation (reviewed in [72]).

While there are challenges in complement testing, there is great potential for this testing to aid in the diagnosis of the increasing number of complement associated disorders identified. With the rapid development of more complement specific therapeutics, testing will only expand, increasing the pressure for more laboratories to be able to perform complement testing. During this phase it may be especially advisable to work with those specialized laboratories that are familiar with this still esoteric field of testing.

Summary

The field of complement therapeutics and diagnostics has entered a new phase of expanded importance in the treatment and diagnosis of human disease. While the first phases of treatment have been limited to rare diseases, the development of additional therapeutics should open up the field to evaluation of inhibitors in more common rheumatic and autoimmune diseases. Successful utilization of existing therapies in case reports of rheumatic diseases, as well as the long history of experimental and clinical association studies illustrating close relationships of complement activation with disease manifestations, bode well for future success in these endeavors.

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Figure 1. Complement System - Overview of its Pathways and Targets of Complement Therapeutics

The three complement activation pathways, and regulatory factors that control them, are illustrated through processes initiated on pathogens by the binding by antibodies and lectin pathway recognition molecules. At upper right, cells are normally protected from these processes; however, when activation is excessive, as lower right, damage ensues and additional downstream inflammatory molecules are generated. Sites where complement therapeutic inhibitors are focused (Table 1) are indicated by "X".

Figure 2. Complement in the Pathogenesis of Experimental Rheumatoid Arthritis

Schematic outline demonstrating roles of complement activators, regulators and cells (adipocytes, fibroblast-like synoviocytes) whose global dysregulation allows pathogenic activation of complement pathways on the cartilage surface, amplification of injury and provision of new proteins that fuel local amplification.

Figure 3. Complement in the Pathogenesis of SLE

The complement system functions both to prevent SLE and as a mediator of tissue injury in the disease. Complement activation facilitates the removal of apoptotic and damaged cells, helping to clear nuclear contents that are released from the injured cells. Complement activation may also help establish tolerance to nuclear antigens. Consequently, deficiencies of various complement components increase the risk of developing SLE. On the other hand, complement activation increases production of type I interferons by dendritic cells and mediates immune-complex-mediated tissue injury.

Figure 4. Pathogenesis of Anti-phospholipid Antibody Syndrome

Anti-phospholipid antibodies bind to $\beta 2$ -glycoprotein 1 ($\beta 2$ -GPI) in complex with phospholipids on the surface of endothelial cells. The antibodies activate the complement cascade, generating C5a and the C5b-9. C5b-9 and C5a cause activation of the endothelial cells, which then express adhesion molecules. Activated endothelial cells also release tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1), creating a hypercoagulable environment. C5a induces monocytes to release soluble vascular endothelial growth factor-1 (sVEGF-1) and neutrophils to release TNF α . Thrombin and fibrinogen can also cause complement activation, perpetuating these pro-inflammatory and hemostatic processes.

Table 1
Diagnostic Approaches to the Complement System

Included are suggested approaches (level, function, genetic) to working up patients with suspected diseases, or to assure coverage of the pathway targeted by therapeutics.

Examples of Complement Inhibitors in Development			
Target	Therapeutic	Developer/Distributor	Phase Achieved in Development/ Indication(s)
C1q	Anti-C1q mAb	Annexon	Pre-Clinical/Neurodegenerative Disorders
C1	C1-INH (Berinert, Ruconest, Cynrizo)	CSL Behring, Salix Pharmaceuticals, Shire	All Approved/HAE
C1s	Anti-C1s mAb	True North Therapeutics	Clinical Development/Cold Agglutinin Disease, Antibody-Mediated Hemolytic Anemia
MASP-2	Anti-MASP-2 mAb	Omeros	Clinical Development/aHUS
MASP-3	Anti-MASP-3 mAb	Omeros	Pre-Clinical
Factor D	Anti-Factor D mAb (Lampalizumab) ACH-4471	Genentech Achillion	Clinical Development/AMD PNH
Factor B	Factor B siRNA Anti-Factor B mAb	Ionis Novemed/Alexion	Pre-Clinical
Properdin	Anti-Properdin mAb	Novemed	Pre-Clinical
C3/C5 Convertases	Compstatin/Derivatives sCR1/ TP10 Mini-FH Mirococept CR2-Factor H/TT30	Apellis, Amyndas Celldex Amyndas AdProTech Alexion	Clinical Development/PNH, aHUS, C3 Glomerulopathy, Renal Transplantation, AMD
C5	Anti-C5 mAb	Eculizumab	Approved/PNH, aHUS
C5 - Follow On	Biosimilar mAbs Coversin/ OmCI RA101495 ALN-CC5 ARC1905 ALXN 1210	Multiple Akari RaPharma Alnylam Ophotech Alexion	Clinical/PNH, aHUS, AMD, Myasthenia Gravis
C5a/C5aR	CCX-168	Chemocentryx	Clinical
C3a/C3aR	Various	Undisclosed	Pre-Clinical
C6	Anti-C6 mAb C6 Anti-sense RNA	Regenesance	Pre-Clinical

Included are examples of complement therapeutics that are under pre-clinical development, clinical development or are approved. Due to the large number of rapidly evolving development programs, as well as the expanding list of indications that are under consideration, this list should not be considered complete but is rather illustrative of key features of the complement therapeutic pipeline. Additional molecular entities are described in reference #26.

Table 2**Complement Diagnostic Testing**

Complement Testing in Diagnosis			
Disorder	Recommended Serum tests	Recommended Genetic test	Considerations
PNH	CD55 & CD59 by FACSCan, Acid lysis test		This is a somatic level mutation so genetic testing is not appropriate
aHUS	CH50, AH50 or ELISA style equivalents, sC5b-9, C3, Factor B, Factor H, Factor I, Factor H Function, anti-Factor H antibodies, CD46 by FACSCAN	CFH, CFI, C3, FB, CD46, FHR1-5, MCP, DGKE, THBD, ADAMTS13	
HAE	C1-INH level & Function C4, C1q, anti-C1-INH antibodies	Serpin-G1, Factor XII	
AMD	Potential for Activation Fragment Testing TBD		This is a developing area and the ability of circulating levels of complement to predict level in the eye is still in development
RA	C3, C4 & CH50	MBL2 testing has been proposed but remains unproven *	
Osteoarthritis	sC5b-9 (soluble Terminal Complement Complex), has been suggested		
SLE	CH50/ELISA CP C1q Level, C3 C3a/ C3b, sC5b-9, anti-C1q-antibody	C4 (C4A/C4B) Halplotypes *	CP-CAP testing is also under consideration for SLE diagnosis
Vasculitis	sC5b-9, C3a, Bb, & C5a have been suggested, C1q auto-antibodies in HUVS		
Complement Testing to Follow Treatment			
Drug Class	Recommended Current Serum Testing	Considerations	
Terminal Pathway Inhibitors	AH50, sC5b-9, C3a	Assessment of alternative pathway function is recommend over classical pathway function due to the ability to test serum at a closer to physiological dilution	
Classical Pathway Inhibitors	CH50, C4a		
Alternative Pathway Inhibitors	AH50, Bb or Ba		

All testing listed, with the exceptions of those listed with a '**', were available at the time of publication from at least one clinical laboratory. Many of these tests will require a laboratory specializing in complement testing. The exact laboratories and their test menus is subject to change so it is recommended that one consult their send-outs department or similar resources.