Complement system is a major effector system of the innate immunity that bridges with adaptive immunity. The system consists of about 40 humoral and cell surface proteins that include zymogens, receptors and regulators. The zymogens get activated in a cascade fashion by antigen-antibody complex, antigen alone or by polymannans, respectively, by the classical, alternative and mannose binding lectin (MBL) pathways. The ongoing research on complement regulators and complement receptors suggest key role of these proteins in the initiation, regulation and effector mechanisms of the innate and adaptive immunity. Although, the complement system provides the first line of defence against the invading pathogens, its aberrant uncontrolled activation causes extensive self tissue injury. A large number of humoral and cell surface complement regulatory protein keep the system well-regulated in healthy individuals. Complement profiling had brought important information on the pathophysiology of several infectious and chronic inflammatory disorders. In view of the diversity of the clinical disorders involving abnormal complement activity or regulation, which include both acute and chronic diseases that affect a wide range of organs, diverse yet specifically tailored therapeutic approaches may be needed to shift complement back into balance. This brief review discusses on the complement system, its functions and its importance as biomarkers and therapeutic targets for autoimmune diseases with focus on SLE and RA.

Keywords: Autoimmune diseases, Complement receptor type 1 (CR1), Decay accelerating factor (DAF), Inflammation, Mannose binding lectin (MBL) pathway, Membrane cofactor protein (MCP), Membrane attack complex (MAC), Polymannans, Rheumatoid Arthritis (RA), Systemic lupus erythematosus (SLE).

Complement was identified more than 100 years ago as a result of its ‘complementary’ bactericidal activity and its role in phagocytosis of cellular debris. This pro-inflammatory system consists of about 40 proteins in the form of inactive enzyme precursors (zymogens), soluble or cell-bound regulatory proteins and receptors specific for effector peptides of the system. These effector peptides are generated by activation of the zymogens in the system by three different pathways triggered by different molecular patterns like antigen-antibody complexes, altered self antigens and pathogen associated molecules. Active peptides and a membrane attack complex (MAC) are generated which effect a wide range of functions including injurious effects to self. Complement regulatory proteins keep complement activation under control and prevent complement mediated injury to self in normal health.

Several studies including ours have suggested that the levels of active complement peptides and expression of complement regulatory proteins get altered in autoimmune diseases and many other disease conditions. This has formed the basis of exploring complement and complement regulatory proteins as biomarkers and therapeutic targets. While each of the complement components hold promise as biomarkers, research has geared up to explore the suitability of complement regulatory proteins especially, those encoded by regulators of complement activation (RCA) and membrane-bound complement regulatory proteins as biomarkers.

Strategies in the development of complement-based therapeutics range from use of anti-complement antibodies, enhancing complement mediated phagocytosis of undesired elements and pathogens, inhibiting complement mediated entry of pathogens to infect the cells, inhibitory structural analogues,
increasing complement mediated regulation of autoinjury, use of DNA mimetics, etc\textsuperscript{5,6}.

**Mechanisms of complement activation**

Activation of the complement pathways occurs in a sequential manner by proteolytic cleavages of zymogens followed by their association\textsuperscript{7}. Complement activation can take place by three different pathways, depending on the molecular triggers. These are; Antibody-dependent classical pathway\textsuperscript{8} antibody-independent alternative pathway\textsuperscript{9} and mannose binding lectin (MBL) triggered pathway\textsuperscript{10}. Overview of the complement activation pathways are depicted in Fig. 1.

All the pathways converge at C3-convertase, resulting in the formation of pro-inflammatory anaphylatoxins (C3a and C5a), chemotaxins (C5a), opsonins (C3b, C4b, iC3b, C3d, C3dg) and a terminal lytic complex C5-C9\textsubscript{(n)}, known as membrane attack complex (MAC)\textsuperscript{11,12}.

**The classical pathway**

The classical pathway is initiated by antigen-antibody complexes containing complement fixing IgG or IgM antibodies and by other molecules generated as a result of an inflammatory reaction such as C-reactive protein or serum amyloid protein\textsuperscript{8,10,13}. This pathway can also be activated in an antibody-independent manner by viruses and gram-negative bacteria\textsuperscript{1}, and its components are named as C1-C9. Activation of the classical pathway is initiated by binding of C1q, a part of the first component of complement (C1), to the Fc portions of immunoglobulins that further activates the C1r and C1s esterases, the subcomponents of C1. C1s esterase cleaves C4 and C2 to form bimolecular complex C4b2a which has C3 convertase enzymatic activity. The C3 convertase cleaves C3 and generates a small fragment C3a and a large fragment C3b. Attachment of C3b to C4b2a complex forms the C4b2a3b complex, which has C5 convertase activity\textsuperscript{10}.

**The alternative pathway**

The alternative pathway is triggered by carbohydrates, lipids and proteins found on foreign and non-self surfaces\textsuperscript{9,14}. It provides a rapid defense against certain pathogens\textsuperscript{14}. C3 is constantly hydrolyzed at a low level (“tick over”) to form C3b, which binds to targets such as bacteria. Factor B is then recruited to the bound C3b followed by Factor D that cleaves Factor B to form the C3 convertase C3bBb, which is stabilized by the presence of plasma properdin\textsuperscript{15}. The spontaneously generated C3 convertase C3bBb cleaves C3 to produce more C3b autcatalytically and form C5 convertase C3bBbC3b. Thus, alternative pathway is devoid of C1, C2 and C4 but has Factor B, D and also P as additional components.

**The mannose binding lectin (MBL) pathway**

The MBL pathway is activated when either mannose binding lectin or Ficolin bind to carbohydrate moieties on surfaces of pathogens.

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**Fig. 1**—Pathways of complement activation. [The classical, MBL and alternative pathways converge into a final common pathway when C3 convertase (C3 con) cleaves C3 into C3a and C3b. Ab (antibody); Ag (antigen); MAC (membrane attack complex); MASP (MBL-associated serine protease); MBL (mannose-binding lectin)]
including yeast, bacteria, parasites and viruses\textsuperscript{11}. It has all the components of the classical pathway except C1 which is replaced by MBL or Ficolin. Both, MBL and Ficolin circulate in the serum as complexes with MBL associated proteins (MASPs). Binding to pathogens induces conformational changes resulting in auto-activation of MASPs which cleaves C4 and C2 to form C3 convertase C4bC2a\textsuperscript{16}. The rest of the cascade is identical to that of the classical pathway.

**Membrane attack complex (MAC)**

Amplification of either of the pathways leads to generation of C5 which then binds sequentially to C6-C9 components to form membrane attack complex (MAC). Binding of C6 to C5b induces a conformational change in C6 making it capable of binding C7. The C5b-7 complex is hydrophobic and capable of binding to lipid membranes. C8 in turn may bind either to a cell-bound or a soluble C5b-7 complex. The C5b678 complex creates a small pore- (10 Å dia) that can lead to the lysis of red blood cells but not of nucleated cells. In the final step, 10-17 molecules of C9 bind and polymerize to form a single C5b678 complex. During polymerization, the C9 molecules undergo transition, so as to insert into the membrane\textsuperscript{17}. The intermediate complexes C5b-7, C5b-8 and C5b-9 formed during the pore assembly are referred to as terminal complement complexes (TCCs), while C5b-9, the final complex and the most effective at inducing cell death, is referred to as the membrane attack complex (MAC)\textsuperscript{17}. The completed MAC has a tubular form and functional pore size of 70-100 Å. Through the central channel of MAC, ions and small molecules can freely diffuse. The cell is unable to maintain its osmotic stability and is killed by an influx of water and loss of electrolytes\textsuperscript{18}. When the number of channels assembled on the surface of nucleated cells is limited, sublytic MAC can activate target cells (neutrophils, endothelial and epithelial cells) to secrete inflammatory mediators, such as reactive oxygen metabolites, prostaglandins and thromboxanes. Sublytic C5b-9 protects cells from apoptotic cell death through post-translational regulation of Bad which inhibits the mitochondrial pathway of apoptosis. Also, C5b-9 inhibits caspase-8 activation; downregulates FasL expression, Bid cleavage and induces significant increase in FLIP cleavage\textsuperscript{19}.

**Peptides generated during complement activation and functions of the complement system**

Complement activation via all the three pathways leads to generation of small anaphylotoxins C3a, C4a and C5a. These anaphylotoxins target a broad spectrum of immune and non-immune cells. Also they act as chemo-attractants of the leukocytes. C5a is the most potent anaphylotoxin and chemotaxin. These peptides trigger inflammatory pathways by interacting with their receptors on the granulocytes and other cells. C3b and C4b are opsonins that facilitate phagocytosis of pathogens, other danger signals and clear immune complexes. The terminal complement complex/(MAC) pores holes on the cell membranes and lyse the cells\textsuperscript{16,20,26}.

In addition, Complement proteins play an important role in modulating adaptive immunity and in bridging innate and adaptive responses\textsuperscript{27}. Complement regulates CD4+ and CD8+ T cell activation and effecter functions\textsuperscript{28}. C3d or C3dg coated immune complexes bind to CR2-CD19-CD81 co-receptor and decrease the threshold required for B cell activation\textsuperscript{29}. In addition, CR2 enhances B -cell memory and CR1 regulates B cell responses. CD46 has been found to regulate the proliferation and effecter functions of CD4+ and CD8+ T cells\textsuperscript{30}. C3a and C5a via their receptors C3aR and C5aR on dendritic cells, T cells and macrophages also influence various adaptive immune responses (Table 1)\textsuperscript{31-34}.

**Complement regulatory proteins—Complement activation is under stringent control by a large number of complement regulatory proteins** (Table 2).

<table>
<thead>
<tr>
<th>Table 1: Complement Peptides and their functions</th>
<th>Complement peptide</th>
<th>Functions</th>
<th>Complement Receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3a,C5a</td>
<td>Anaphylaxis and chemotaxis, provokes inflammation, regulation of adaptive immunity.</td>
<td>C3a,C5a receptors on granulocytes, dendritic cells, T cells and macrophages.</td>
<td></td>
</tr>
<tr>
<td>C3b,C3bi,C4b</td>
<td>Opsonization, facilitation of phagocytosis, ADCC, clearance of immune complexes, regulation of adaptive immunity, facilitate entry of certain opsonized pathogens like M. tb.</td>
<td>CR1, CR3, CR4, CR3 and CR4 are integrins.</td>
<td></td>
</tr>
<tr>
<td>C3d</td>
<td>Induction of B-cell responses.</td>
<td>CR2 on the B-lymphocytes</td>
<td></td>
</tr>
<tr>
<td>C5b6789(n), MAC</td>
<td>Poke holes into the bacterial cell membranes and lysis of the cell, Induction of apoptosis.</td>
<td>Plasma membrane lipid bi layer.</td>
<td></td>
</tr>
</tbody>
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Table 2—complement regulatory proteins

<table>
<thead>
<tr>
<th>Regulator/soluble/membrane-bound/Pathway</th>
<th>Mechanism of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 inhibitor (C1Inh)/soluble/Classical Pathway</td>
<td>Inhibits Serine protease, dissociating C1r2s2 from C1q.</td>
</tr>
<tr>
<td>C4 BP</td>
<td>Binds with C4b and inhibits the formation of C3 convertase.</td>
</tr>
<tr>
<td>Factor H(FH)/soluble/Alternative Pathway.</td>
<td>Binds with C3b; cofactor for cleavage of C3b by factor I. Inhibits formation of C3 convertase. Fluid phase inhibitor.</td>
</tr>
<tr>
<td>Factor-I /soluble</td>
<td>Serine protease: cleaves C4b or C3b using C4bBP, CR1, factor H, DAF, or MCP as cofactor</td>
</tr>
<tr>
<td>Serum proteases/soluble S protein/ Vitronectin/soluble Anaphylatoxin inactivator/soluble</td>
<td>Blocks fluid-phase MAC</td>
</tr>
<tr>
<td></td>
<td>Blocks soluble C5b67 and prevents its insertion into cell membrane</td>
</tr>
<tr>
<td></td>
<td>Inactivates anaphylatoxin activity of C3a, C4a, and C5a by carboxypeptidase N removal of C-terminal Arg</td>
</tr>
<tr>
<td>Membrane-cofactor protein (MCP)/membrane-bound/Classical, alternative and lectin pathway</td>
<td>Block formation of C3 convertase by binding C4b or C3b; cofactor for factor I-catalyzed cleavage of C4b or C3b C3bBb</td>
</tr>
<tr>
<td>Decay-accelerating factor (DAF or CD55)/membrane-bound/Classical, alternative and lectin pathway</td>
<td>Accelerates dissociation of C4b2a and C3bBb(classical and alternative C3 convertases)</td>
</tr>
<tr>
<td>Membrane inhibitor of reactive lysis (Protectin/HRF20/ MIRL or CD59)/ membrane-bound</td>
<td>Bind to C5b678 on autologous cells, blocking binding of C9</td>
</tr>
</tbody>
</table>

The regulation is achieved either by intervening the cascade amplification by binding proteins (C4bp, C3bp), inhibiting the activation (C1 inhibitor), inhibiting the formation of the end MAC (CD59, s-protein, clusterin), accelerating the decay of the active fragments (DAF, CR1) and acting as a cofactor (CR1, MCP) for the ultimate destruction of active peptides by factor I, a serine protease. Factor I is the master regulatory protein as it degrades the active complement fragments in no time with the help of the co-factors and decay accelerating proteins. Most stringent regulation is at the level of C3 or C4 by a group of complement regulatory proteins encoded by a single gene cluster called regulators of complement activation (RCA) gene family.

Complement as biomarker—With regard to human disease, the complement system has been shown to play a role in the pathogenesis of various immune-mediated disorders, including dermatologic, renal, neurologic, and rheumatic diseases.

Complement and complement regulatory proteins as biomarkers for RA and SLE

Exaggerated complement activation, deficiency of complement components or modulation of complement regulatory proteins had been observed in autoimmune disorders. While active complement peptides and MAC may cause significant cellular injury in autoimmune disorders, complement regulatory proteins protect the host against complement mediated autoinjury.

Rheumatoid arthritis (RA)

There is increasing evidence that the complement system could play an important role in the pathogenesis of RA. Complement activation could be induced by immune complexes consisting of type II collagen (CII) and CII autoantibodies or by IgG and rheumatoid factor in vitro. Similar mechanisms are activated by increased release of histamine in response to C5a by synovial mast cells, which have been shown to bear significant amounts of C5aR in RA compared with OA. Assembly of TCC on cell membranes results in the formation of, which can lead to lytic attacks on synovial cells and chondrocytes, facilitated by low levels of the membrane-associated inhibitor of the MAC protectin (CD59) on synovial cells MAC. Since synovial cells as well as other nucleated cells are rather resistant to lytic attacks, the primary effect of the MAC in RA appears to be sublytic attacks on the cells. It results in a release of reactive oxygen metabolites, prostaglandin E2, leukotriene B4, and interleukin-6 from synovial cells which may lead to enhanced DNA damage in rheumatoid synovium and increased collagenase specific mRNA expression by synovial fibroblasts. These effects are supported by low concentrations of the fluid-phase MAC inhibitors clusterin and vitronectin in synovial fluid. Of interest, the “hot zone,” in which complement activation results in actual damage, appears to be the microvasculature, a fact that is illustrated by the intensive amounts of C5b-9 close to activated lymphocytes in this area. Certain complement proteins may also contribute to joint destruction. C1s, which is increased by TNF in chondrocytes, is involved in cartilage degradation. More important, complement, in particular C1s in conjunction with matrix metalloproteinase 9, has a physiologic role in remodeling of cartilage in the
growth plate and in fracture healing. Findings in studies using animal models of RA also support the hypothesis that complement is required for the development of arthritis. In DBA/1LacI mice with collagen-induced arthritis (CIA), systemic administration of a specific anti-C5 monoclonal antibody inhibits terminal complement activation in vivo and prevents the subsequent on set of arthritis in immunized mice, and ameliorates established disease. Wang and colleagues also concluded that activated TCC plays an important role in the induction as well as the progression and manifestation of disease. Moreover, C5-deficient mice do not develop CIA.

Many studies have shown high levels of complement components and active complement metabolites C2, C3, C3a, C5a, and TCC C5b–9 in serum, SF, and ST. Furthermore, positive correlations have been shown between SF complement activation (e.g., levels of plasma C3dg, a C3 activation metabolite) and local as well as general disease activity in RA and between C2 and C3 expression in RA synovium and inflammation.

Systemic lupus erythematosus (SLE)

Measuring serum complement levels had been considered the "gold standard" for monitoring disease activity in SLE patients. Six decades ago, Vaughan et al. revealed the association between decreased complement and active SLE. On usefulness of these assays in monitoring SLE, some studies have concluded that measuring C proteins are valuable for predicting the disease course while other studies have deduced that measuring C proteins are of minimal value. Conventionally, complement activation is assessed by functional assays quantifying hemolytic activity (CH100 or CH90 which measure total complement activity) or by static measures of serum complement components such as C1q, C2, and C4 (classical pathway activation), factor B (alternative pathway activation), or C3 (representing the common terminal pathway). Low concentrations of complement components due to increased catabolism are found in a majority of patients with active and severe SLE. The sensitivities and specificities for the tested components were 70 and 70%, respectively, for CH50, 64 and 91% for C3 and 64 and 65% for C4. In further analysis by recursive partitioning on the same data set, the combined sensitivity and specificity for CH50, C3 and C4 were 74 and 88%. In a more recent study, low levels of C1q were found to have a high specificity for SLE (96%) but the sensitivity was low (20%). However, according to some studies, measurement of complement proteins in the plasma doesn’t provide a reliable indication of the disease activity. This is because apart from the disease process, level of C also varies due to difference in the rates of synthesis among patients. In an effort to improve the value of complement measurements in the assessment of disease activity, attempts have been made to measure the levels of serum markers of complement activation, such as anaphylatoxin levels, C1r-C1s-C1 inhibitor complexes, C4d levels, iC3b or C3dg levels and MAC/TCC levels to replace the static measurement of complement proteins. Such measurements are more sensitive than regular measurement of CH50 or complement components such as C1q, C4 and C3. None of these assays has been widely adopted in clinical practice. Each of these complement activation products is highly unstable in vivo and, therefore, difficult to measure with any useful reliability outside a research setting making them unsuitable for clinical diagnosis. Moreover, antibodies to complement protein, especially C1q, can cause rapid complement activation, which does not reflect disease activity. Some reports state that markers of complement activation in tissues correlate better with the presence of inflammation, e.g., the presence of the membrane attack complex is more prominent in inflamed tissues like kidney or skin compared with clinically normal tissues from patients with SLE.

Proliferative glomerulonephritis (WHO class III and IV) and that C1q concentration decreased prior to clinical manifestations of flares of the disease. Low C1q levels have also been shown to predict the histopathological outcome of lupus nephritis. C3d measurement in urine has also been suggested to be of value for evaluation of active SLE glomerulonephritis. In sequentially followed patients, evidence of activation of the early part of the classical pathway measured by C1NH2-C1r-C1s complexes is detected prior to C3 activation. For a better predictive value, sequential measurements of C are required for monitoring disease activity.

Since complement activation occurs in several co-morbidities of SLE, especially infections, the specificity of C level as a disease marker is low, even though the assays well discriminate active disease from the remission state. Furthermore, artefacts caused by complement activation in vitro during coagulation and other handling procedures add to the problem.
Membrane-bound complement regulatory proteins as biomarkers

Studies with gene knockout mice have suggested that membrane bound complement regulatory proteins may critically determine the sensitivity of host tissues to complement injury in autoimmune and inflammatory disorders. The upregulation of many of the membrane bound molecules have been reported in inflammatory tissues and organs affected by autoimmune diseases. In vitro studies have also revealed that the expression of the CRPs can be modulated by ICs, several cytokines like TNF-alpha, IL-1beta, TGF-beta, and IFN-gamma, etc. The membrane-bound complement regulatory proteins decay-accelerating factor (DAF, CD55), membrane cofactor protein (MCP, CD46), complement receptor-1 and CD55 provide protection from autologous complement-mediated injury. DAF and MCP act at the level of the C3 convertase. In contrast, CD59 inhibits the terminal pathway of complement activation, preventing the incorporation of C9 into the MAC.

The most studied complement regulatory protein is CR1. Role of other CRPs like C1 inhibitor, DAF, MCP, CD59 and others are beginning to emerge. Following is a brief account of the studies those suggest potential of CR1, DAF, MCP and CD59 as the biomarkers for RA and SLE.

Complement receptor type 1 (CR1, CD35)

Rheumatoid arthritis (RA)—It had been observed that CR1 expression goes down on erythrocytes and leukocytes. The reduced level of CR1 on these cells is speculated as a key mechanism contributing to immune complex overload and exaggerated complement activation in RA. Existing reports have indicated that CR1 has a negative regulatory role in the activation of human B and T lymphocytes. The underlying mechanism is not clear. Several studies have documented modulation of CR1 levels in RA. Lowered expression of E-CR1 has been reported in a number of studies. However, studies on leukocytes have reported increased expression of CR1.

In view of the exaggerated complement activation in RA and significance of complement receptor 1 (CR1/CD35) as a complement regulatory protein (CRP), this author observed lower levels of leucocyte-complement receptor 1 (L-CR1) transcript in 45 RA patients compared to 66 controls, and their correlations with the levels of CIC, C3d and DAS28 at different time-points in RA patients suggested CR1 as a potential disease marker for RA.

Systemic lupus erythematosus (SLE)—Walport and colleagues observed low CR1 and deposition of C4 and C3 fragments on erythrocytes in active SLE. A later study demonstrated that the combined detection of high levels of erythrocyte-bound C4d and low levels of CR1 on erythrocytes had high sensitivity (72%) and specificity (79%) for SLE. E-CR1 got much attention due to the fact that E-CR1 plays an important role in the clearance of immune complexes, overload of which is a major cause of the pathological manifestations in SLE. In addition to E-CR1, lower levels of CR1 on leukocytes and glomerular podocytes were also reported in SLE. Other Studies have documented a decline in B cell and neutrophil CR1, which correlated positively with the decline in E-CR1 in SLE. Another study showed that recombinant sCR1 could restore the immune complex binding ability of CR1-deficient erythrocytes. These findings strongly suggested a key role of deficient CR1 expression in the pathogenesis of SLE.

Further studies from our lab showed a drastic decline in the levels of u-CR1 in SLE which correlated with expression of glomerular CR1. Therefore, u-CR1 was suggested as a potential marker to diagnose glomerular involvement in SLE. Earlier, we observed E-CR1 to be a prognostic marker for glomerulonephritis and rheumatoid arthritis. Later we found marked decline in the levels of CR1 transcript and protein and negative correlations between the levels of leucocyte CR1 transcript and protein with the disease activity and severity of SLE. This suggested L-CR1 transcript as a disease activity marker for SLE. Further studies are needed in this direction to be more conclusive about the usefulness of leucocyte CR1 as a bio marker for SLE. Membrane cofactor protein (MCP, CD46)

Rheumatoid arthritis (RA)—In RA, in a retrospective and follow-up study, we found a marked decline of neutrophil and PBMC MCP transcript in patients with rheumatoid arthritis which correlated negatively with the disease activity. Studies elucidating the role of MCP in SLE modulation are limited. There is a report of elevated serum levels of soluble MCP in SLE patients with SLE. Also, the level was significantly higher in case of patients with active SLE compared to those with inactive SLE and the level declined with cortisol treatment. Longitudinal analysis of soluble sMCP showed that sMCP levels decreased in parallel with the levels of anti-dsDNA and correlated with reduced CH50 levels. However, the source as well as the function of this serum sMCP is not clear. An
immunohistochemical study of the expression of MCP in the kidney tissues of patients with renal diseases, including lupus nephritis has shown significantly increased intensity of MCP in the affected glomeruli when compared to normal. Since there are a massive deposition of C3 and immunoglobulin in the glomeruli of lupus nephritis patient, the C3 regulatory protein MCP might have a protective role in the glomerular tissue injury and the increased MCP could be a result of up-regulated synthesis of MCP in the glomerular cells.

Systemic lupus erythematosus (SLE)—In another study, we have suggested that the expression of leukocyte MCP at the mRNA level is closely related to disease activity in SLE. Further, we speculated a protective role of MCP in response to increased disease burden. Moreover, the follow-up study suggested MCP as a potential disease marker.

Decay accelerating factor (DAF, CD55)
Not much is known about the role of DAF in the disease process of SLE; however, evidence strongly indicates its possible role in autoimmune disease modulation. A study of the autoimmune disease in DAF knockout MRL (DAFKO MRL/lpr/lgh) mice has shown more aggressive disease development in such mice characterized by accelerated and aggravated dermatitis and lymphadenopathy as well as increased antichromatin autoantibody production. Injection of a subnephritogenic dose of rabbit anti-mouse glomerular basement membrane serum has induced glomerular disease in DAF knockout mice but not in wild-type controls, with DAF knockout mice displaying an increased glomerular volume. Enhanced expression of DAF has been documented in glomerular cells in the presence of glomerular injury indicating an enhanced protective role of this CRP to complement attack. On neutralizing DAF with antibody, the glomerular epithelial cells have been demonstrated to be more susceptible to complement mediated cytotoxicity, which elucidates the functional importance of DAF on glomerular cells. However, there is a contradictory report stating a slight but statistically significant decrease of DAF level on RBCs of SLE patients with diffuse proliferative glomerulonephritis, which was dependent on the stage of the disease activity. Another study reported a deficiency of red cell bound DAF in SLE patients with autoimmune hemolytic anemia, indicating the modulatory role played by DAF in the different clinical manifestations of SLE.

A study from our lab on the level of DAF in patients with Rheumatoid arthritis reported significantly reduced expression of this protein on the erythrocytes and a significant inverse relationship between DAF expression and in vitro complement activation. We observed increased erythrocyte DAF in SLE patients and an enhanced expression of the same in the glomeruli of SLE patients. Also, we found a significant decline in the levels of leukocyte DAF in RA, but increase in SLE with significant correlations with the disease activity.

CD59 (Protectin)
Rheumatoid arthritis (RA)—Expression of CD59 is significantly decreased on the synovial lining, stromal cells and EC. Moreover, injection into the rat knee joint of an anti-rat CD59 mAb induces a spontaneous complement-dependent arthritis and CD59-deficient mice are prone to enhanced antigen-induced arthritis.

Systemic lupus erythematosus (SLE)—Several studies have demonstrated increased serum levels of MAC C5b-9 in active SLE patients indicating the potential role of C-mediated tissue injury in the disease process. Injection of a subnephritogenic dose of rabbit anti-mouse glomerular basement membrane serum has induced glomerular disease in DAF knockout mice but not in wild-type controls, with DAF knockout mice displaying an increased glomerular volume. Enhanced expression of DAF has been documented in glomerular cells in the presence of glomerular injury indicating an enhanced protective role of this CRP to complement attack. On neutralizing DAF with antibody, the glomerular epithelial cells have been demonstrated to be more susceptible to complement mediated cytotoxicity, which elucidates the functional importance of DAF on glomerular cells. However, there is a contradictory report stating a slight but statistically significant decrease of DAF level on RBCs of SLE patients with diffuse proliferative glomerulonephritis, which was dependent on the stage of the disease activity. Another study reported a deficiency of red cell bound DAF in SLE patients with autoimmune hemolytic anemia, indicating the modulatory role played by DAF in the different clinical manifestations of SLE.
studies on the role of CD59 in autoimmunity deal with the expression of CD59 in the kidney of patients with renal diseases.

An increased expression of CD59 on the RBCs of SLE patients with diffuse proliferative glomerulonephritis was also observed in our lab. There is a contradictory report showing decreased level of CD59 on the red cells of SLE patients with autoimmune hemolytic anemia. Thus, the expression of CD59 on erythrocytes may vary with the varied manifestations of SLE. A follow-up study carried by us showed increased levels of CD59 transcript during the active stage of disease that declined on remission and reverted to the first day high levels during flares. Preliminary studies in our lab found a decreased expression of CD59 in leucocytes of the patients with RA at the transcript and protein level. Detailed studies are warranted.

Therapeutic implications

In view of the diversity of the clinical disorders involving abnormal complement activity or regulation, which include both acute and chronic diseases and affect a wide range of organs, diverse yet specifically tailored therapeutic approaches may be needed to shift complement back into balance. A large number of complement therapeutics on the market or in clinical trials. Many more are on development. They hold promise in the treatment of organ transplantation, ischemia-reperfusion injury, coronary artery disease, myocardial infarction, stroke, infection, cancer, immunosuppression, paroxysmal nocturnal hematuria, glomerulonephritis, rheumatoid arthritis, and acute respiratory distress syndrome, and have also been used in the coating of extracorporeal circuits in cardiopulmonary bypass and dialysis.

Strategies in the development of complement-based therapeutics range from use of anti-complement antibodies, enhancing complement mediated phagocytosis of undesired elements and pathogens, inhibiting complement mediated entry of pathogens to infect the cells, inhibitory structural analogues, increasing complement mediated regulation of autoinjury, use of DNA mimetics etc.

The endogenous soluble complement-inhibitors, antibodies or low molecular weight antagonists that either block key proteins of the cascade reaction or neutralize the action of the complement-derived anaphylatoxins have successfully been tested in various animal models over the past years. Promising results consequently led to first clinical trials.

A strategy can be inhibiting initial component of one or more of the activation pathways like blocking the activation of factor D (Alternative pathway), C1q (Classical pathway) or MBL, (Lectin pathway).

Most of the approaches to inhibit complement have focused either on blocking at the level of C3 that implies a general and broad inhibition of the system, or on selective blocking of C5 activation with subsequent inhibition of C5a and C5b-9 (TCC) formation. Another strategy may be neutralizing C3a, C5a or using their receptor antagonists.

Compstatin, a 13-residue cyclic peptide was isolated using combinatorial peptide libraries to identify C3 binding peptides, later characterized in detail and named compstatin. This peptide blocks cleavage of C3 and is highly specific for binding to C3. No interaction with other cascade proteins has been demonstrated. It was found to reduce complement and granulocyte activation in an in vitro model of extracorporeal circulation and to protect against hyperacute xenograft rejection ex vivo. Compstatin works only in primates, and therefore, animal studies are limited. Soulika et al. demonstrated inhibition of heparin-protamine-induced complement activation in a primate in vivo model.

Monoclonal antibodies that specifically inhibit terminal complement activation while preserving the critical functions of the early complement cascade have now been developed. These antibodies target the C5 complement protein, blocking its cleavage and the subsequent generation of potent proinflammatory molecules. Two different anti-C5 single chain Fv (ScFv), one that inhibits both release of C5a and assembly of the TCC (TS-A 12/22) and another that selectively blocks formation of the TCC (TS-A 8), could represent potential therapeutic agents to be used in patients with RA.

Early complement proteins are critical in the clearance of immune complexes and apoptotic bodies, and their absence predisposes individuals to SLE. Alternatively, TCC activation is associated with exacerbations of disease and damage to tissues and organs, particularly in lupus nephritis.

Case-controlled and follow-up studies carried out in our laboratory suggest deficient leukocyte DAF, MCP, CR1, and CD59 expression in RA and deficient E-CR1,G-CR1 and L-CR1 expression SLE. Replenishing these proteins along with administration of anti-C5 may be an effective therapeutic strategy for these diseases. Anti-C5 therapeutics have recently
been investigated both in an animal model of SLE and in humans. Recombinant soluble membrane-bound complement regulatory proteins are promising therapeutic targets for complement mediated inflammatory disorders. Strategy is to target these soluble proteins to the cell membrane or to the specific site to enhance their endogenous activity as complement inhibitors.

Two complement inhibitors, soluble complement receptor 1 (TP10) and a monoclonal anti-C5 antibody (Eculizumab) have been shown to inhibit complement safely and now are being investigated in a variety of clinical conditions. Eculizumab has shown to reduce hemolysis and has been approved by the FDA in paroxysmal nocturnal hemoglobinuria. Although still no clinical trial has been performed in SLE, they hold promise to be used therapeutically in SLE.

Conclusion

With increasing understanding of the role of complement and complement regulatory proteins in inflammatory and autoimmune disorders and literature culminating on RA and SLE in this context, it is clear that many of the cascade proteins especially C1q, C3, C3a, C4, C5, C5a along with TCC hold promise as biomarkers. Among complement regulators, expression levels of membrane-bound complement regulatory proteins especially CR1 and MCP may help in assessing the disease prognosis.

Several small molecules recognized as complement inhibitors, antibodies against initial, central and terminal complement components and receptor antagonists along with various formulations of recombinant soluble CR1 and soluble forms of other membrane-bound complement regulatory proteins are under trial as therapeutics. Prevention of TCC and anaphylatoxin formation and blockade of C3a/C5a receptor rather than blanket immune-suppression may be an effective therapeutic strategy against RA and SLE.

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