Complement in Lupus Nephritis: The Good, the Bad, and the Unknown

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Summary: The complement system consists of 3 pathways and more than 30 proteins, including those with biological activity that directly or indirectly mediate the effects of this system, plus a set of regulatory proteins necessary to prevent injudicious complement activation on host tissue. The role for complement in the pathogenesis of systemic lupus erythematosus (SLE) is paradoxic. On one hand, the complement system appears to have protective features in that hereditary homozygous deficiencies of classic pathway components are associated with an increased risk for SLE. On the other hand, immune complex–mediated activation of complement in affected tissues is clearly evident in both experimental and human SLE along with pathologic features that are logical consequences of complement activation. By using accurate mouse models of SLE, we have gained remarkable insights into pathogenic features likely relevant to the human disease, and the ability to test potential therapies, some of which have made it to standard clinical use. Studies in genetically altered mice and using recombinant protein inhibitors of complement have confirmed what was believed but unproven—early complement proteins C1q and C4 are protective whereas complement activation later in the pathways is proinflammatory and deleterious. Two complement inhibitors, soluble complement receptor 1 (TP10, Avant Immunotherapeutics, Needham, MA) and a monoclonal anti-C5 antibody (Eculizumab, Alexion Pharmaceuticals, Inc., Cheshire, CT) have been shown to inhibit complement safely and now are being investigated in a variety of clinical conditions. Although these and others earlier in their clinical development hold promise to be used therapeutically in lupus nephritis, this optimism must be tempered by the fact that the clinical trials to prove this remain fraught with obstacles.
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The complement system is an important part of innate immunity that defends the host against infectious microorganisms, clears immune complexes (ICs) and dead cells, and connects innate to adaptive immunity.[1-3] Complement can be activated through classic, alternative, and mannose-binding lectin (MBL) pathways shown schematically in Figure 1. Each has different initiators. The classic pathway is activated when C1 binds to immunoglobulin (Ig)M or IgG in ICs. Activated C1 (as the multiprotein $C1qr_2s_2$ complex) cleaves both C4 and C2 to generate C4a/C4b and C2a/C2b. C4b2a acts as a C3 convertase that cleaves and activates C3. The alternative pathway is spontaneously active through hydrolysis of C3, which binds factor B (CFB) in the fluid phase. On cleavage by factor D (CFD, adipsin), an initial C3 convertase is formed that can amplify itself to form C3bBb, the alternative pathway C3 convertase. The binding of MBL to terminal carbohydrate groups on certain microbes leads to the activation of the MBL pathway. Activation of each pathway leads to the cleavage of C3 and C5, with the generation of the C3a and C5a anaphylatoxins, C3b opsonins, and C5b to start the nonenzymatic assembly of the C5b-9 membrane attack complex.

The main effectors of the complement system act through specific cellular receptors: the

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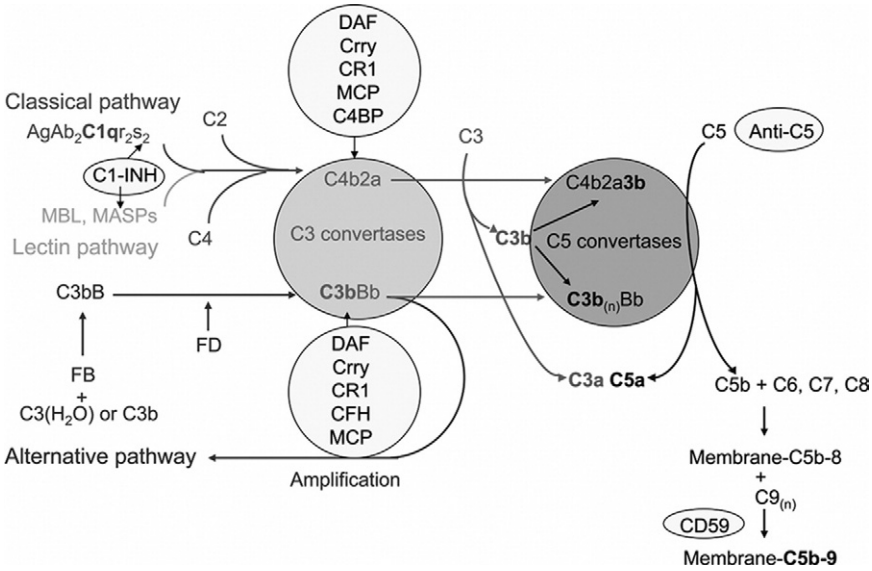

Figure 1. The complement system. Shown are the 3 activation pathways: classic, MBL, and alternative pathways, and the common intermediates of activation, C3 and C5 convertases. The main effectors of complement's actions, C1q, C3a, C3b, C5a, and C5b-9, are in bold type.

G protein–coupled C3a and C5a receptors (C3aR and C5aR) and complement receptors 1-4 (CR1-4) for C3b, iC3b, and C3dg. The latter 2 are specific products of factor I (CFI), which requires a cofactor from among CR1 (CD35), factor H (CFH), and membrane cofactor protein (CD46). C1q also has important biological effects. The cell surface protein CD93 was once considered to be a specific cellular C1q receptor (C1qR),[4] which more recently was disproved[5]; the roles for other C1qRs, including globular C1qR (gC1qR) and calreticulin (cC1qR), still are under investigation.[6] Although these various receptors are traditionally on effectors of the immune system such as neutrophils and monocyte/macrophages, there is growing appreciation for effects on other immune cells, such as B and T lymphocytes, follicular dendritic cells, and what can be considered local organ-specific immune system. For example, in the kidney, epithelial cells of the glomerulus and proximal tubule bear C3aR and CR1.[7-9] In contrast to the specificity of cellular receptors for C3a, C3b, and C5a, C5b-9 is promiscuous in its cellular binding, requiring simply a receptive lipid bilayer for insertion.[10] C5b-9 can result in cellular death by its insertion, which occurs in *Neisseria* sp bacteria[11] and erythrocytes (E) in paroxysmal nocturnal hemoglobinuria,[12] as well as recruit and activate specific signaling cascades in nucleated cells to result in a number of cellular events.[13-15]

Given the potency of the complement system, natural fluid-phase and cell membrane–bound regulatory proteins acting throughout the 3 cascades are essential to prevent activation and injury to host tissues (Fig. 1).[1,2] The regulators of complement activation gene family on human chromosome 1q3.2 (and a comparable location in mouse chromosome 1) includes membrane cofactor protein, CR1, decay accelerating factor (DAF, CD55), C4b-binding protein, and CFH.[16,17] These proteins inhibit complement activation through interactions of their conserved short consensus repeats with fragments of C3 and/or C4.[18] Specific to rodents is the 65-kd rodent complement regulatory protein, p65,[19] more commonly referred to as *CR1-related gene/protein y* (Crry) because of its protein and nucleotide similarity to human CR1.[20] Although CR2 (CD21) is a regulator of complement activation member, it has the highest affinity for C3dg and does not inhibit C3 convertases. Similarly, the β_2 integrins CR3 (*Itgam*, CD11b/CD18, and Mac-1) and CR4 (*Itgax* and CD11c/CD18) have binding affinity for iC3b and also are not complement inhibitors.[21] At the ends of the complement

cascades are C1-inhibitor (C1-INH) and CD59, which inhibit C1 activation and C5b-9 formation, respectively.[22,23]

The involvement of the complement system in the pathogenesis of a number of autoimmune diseases is well accepted, yet its exact roles still are not clear. On one hand, hereditary deficiencies of early classic pathway complement components predispose patients to systemic lupus erythematosus (SLE). On the other hand, activation of complement by ICs is certainly a prominent feature in SLE. Therefore, an imbalance of the complement system in either way can be relevant to the development of this disease. In this review we discuss the dual roles of complement in SLE, and in particular in lupus nephritis. The relevance of what we now know of this system to potential treatment of human SLE is emphasized.

WHY COMPLEMENT IN HUMAN SLE?

Initially, complement caught people's attention based on the observations of hypocomplementemia in patients with active SLE. Low total complement hemolytic activity and decreased C3 and C4 levels have been found in about 75% of SLE patients with focal nephritis and in 90% of patients with diffuse nephritis.[24] The colocalization of immunoglobulin isotypes IgG, IgA, and IgM with C1q, C4, and C3 (and C5b-9) is called a *full house*, which is present almost exclusively in glomeruli of patients with lupus nephritis.[25] Complement split products such as C3d and C5b-9 also can be detected in the urine of SLE patients and provides further circumstantial evidence that complement is involved in lupus nephritis.[26]

Impairment of IC handling also is believed to be pathogenic and relevant to the complement system in SLE patients.[27,28] ICs can form in glomeruli through the passive trapping of preformed circulating ICs and/or the binding of plasma antibodies directly with intrinsic or extrinsic antigens in glomeruli.[29] Because the complement system is required at all steps of normal IC metabolism, any number of alterations can lead to pathologic glomerular IC accumulation, particularly in conditions of IC excess, as in SLE.[27,30] The first step is classic pathway activation on ICs, leading to incorporation of C4b and C3b, which can profoundly affect the physicochemical characteristics of both circulating and tissue-bound (glomerular) ICs.[28,31,32] The next key complement protein is CR1, present on Es (or CFH on the rodent platelet[33]), where it has binding affinity for C3b, and serves as a CFI cofactor for the cleavage of C3b to iC3b. ICs bound to human E CR1 (or mouse platelet CFH) are transported and transferred to cells of the mononuclear phagocyte system such as liver or spleen. The ICs are removed and the Es return to the circulation. Studies have shown the association of low levels of E-CR1 with SLE,[34,35] suggesting that a defective E/IC-clearing system may be related to SLE pathogenesis. Glomerular podocytes also bear CR1, which may play a role in IC processing locally and is decreased similarly in lupus nephritis.[7]

As a paradox to the widespread belief that generation of complement activation products in kidney and other disease sites is proinflammatory, patients with homozygous deficiencies of the C1 proteins (C1q or C1r/s) or C4 have a high prevalence (>80%) of autoantibodies and SLE-like disease.[36,37] Despite the strongest association of C1q deficiency with SLE, because more than 90% of such patients can develop disease features, neither heterozygous deficiency of C1q nor single-nucleotide polymorphism in *C1qA* leading to reduced C1q levels are associated with an increased risk for lupus nephritis.[38] It also is noteworthy that autoantibodies to C1q and co-existent hypocomplementemia also are associated with lupus nephritis, potentially by amplifying complement activation locally.[39,40] Interestingly, homozygous deficiency of C3, the most critical protein that affects all 3 pathways of complement activation, is not associated with SLE.[41] The finding that homozygous deficiency of early components of the classic pathway other than C3 predispose to SLE suggests that physiologic function of these molecules are protective in SLE. Although the exact mechanism is still unknown, one very plausible explanation is the clearance hypothesis proposed by Walport et al.[42] In their well-designed studies, they found that mice with generated C1q and C4 deficiencies had impaired ability to clear apoptotic debris,[42] leading to the accumulation of

potentially immunogenic autoantigens and initiation of an autoimmune reaction in the right genetic setting. In further support of this, C1q-deficient mice had increased mortality and higher titers of autoantibodies, with 25% of the mice developing glomerulonephritis, characterized by glomerular IC deposits and apoptotic debris.[43]

MOUSE MODELS OF HUMAN SLE

There are several murine models that spontaneously develop lupus-like syndromes. Two of the best studied models are the F_1 cross between New Zealand Black and New Zealand White mice (NZB/W) and the MRL/MpJ-*Tnfrsf6*$^{lpr/lpr}$/J (MRL/*lpr*) strain.[44] Similar to the female predominance in human beings, only female NZB/W mice develop SLE. MRL/*lpr* mice are on the autoimmune MRL/Mp background with a retrotransposon in the *Tnfrsf6* (*Fas*) gene leading to nearly complete absence of the proapoptotic Fas protein.[45] Both models have B-cell hyperactivity, autoantibodies, hypocomplementemia, circulating and glomerular-bound ICs, and severe nephritis. As in human beings, there is plenty of circumstantial evidence that complement activation is involved actively in the pathogenesis of glomerular disease in these mice. In early stages (4 and 5 months in MRL/*lpr* and NZB/W mice, respectively), granular deposition of mouse IgG, IgA, IgM, and C3 are present largely in the mesangium, coincident with histopathology showing mesangial proliferation. As the disease progress, there are glomerular capillary wall IC deposits, proliferation of intrinsic endothelial and mesangial cells, and infiltration with inflammatory cells. Eventually, crescent formation (more often in MRL/*lpr* mice) and glomerulosclerosis (more often in NZB/W mice) occur, and mice die of renal failure.[44]

Other spontaneous mouse models (eg, BXSB and congenic strains derived from New Zealand mice[46]), and those induced by antibodies[47] or with deficiency of certain genes such as transforming growth factor-β1,[48] have been generated and studied. These models are not discussed in this article because they are used less frequently in complement research. MRL/Mp mice with normal Fas protein (MRL/Mp$^{+/+}$) or animals of the nonautoimmune C57BL/6 background with the *lpr* gene (C57BL/6$^{lpr/lpr}$) can be considered autoimmune prone, and are useful to test whether a given alteration accelerates autoimmunity. For instance, deficiency of C1q in MRL/Mp$^{+/+}$ mice hastens the development of SLE disease features.[49]

FUNCTIONAL STUDIES OF COMPLEMENT IN EXPERIMENTAL LUPUS MODELS

The manipulation of individual complement proteins through genetic techniques in lupus mouse strains has provided considerable insight into how complement is involved in this disease. In addition, functional inhibition through the use of specific antibodies or antagonists using recombinant or transgenic techniques can be extremely illuminating. Given that C3 is the common point connecting all 3 pathways in complement activation, there are more naturally occurring proteins regulating its activation than anywhere else. As such, many of the studies in lupus mice performed to date have concentrated on activators and regulators of C3.

DAF is a ubiquitously expressed glycosylphosphatidylinositol-anchored membrane protein that inhibits C3 activation through all pathways by inhibiting formation and accelerating decay of the C3/C5 convertase.[50] Histochemically, DAF is present mainly in the juxtaglomerular apparatus.[51] It is notable that human, rat, and mouse podocytes appear to have functional DAF, as shown through a variety of in vivo and in vitro experiments.[52-54] The relevance of DAF to kidney diseases is suggested by enhanced expression in mesangial cells, tubular cells, vascular endothelium, and infiltrating inflammatory cells in disease states.[51,55,56] The Song group showed that DAF-deficient MRL/*lpr* mice had exacerbated lymphoproliferation, antichromatin autoantibody production, and dermatitis, which was particularly evident in females, whereas lupus nephritis appeared to be unaffected.[57] Overall, it seems likely that DAF is not the most important glomerular complement regulator in lupus nephritis, given the co-existent strong expression of Crry in mouse glomeruli, which could compensate for these instances in which DAF was deficient. The exacerbated dermatitis can be explained by the fact that DAF is expressed strongly in the skin whereas Crry is not. The finding of enhanced

autoimmunity in DAF-deficient MRL/*lpr* mice, together with the fact that DAF is also a ligand for the activation-associated lymphocyte antigen CD97,[58] also suggests that DAF may function as a negative regulator of adaptive immunity. This intriguing hypothesis was supported by the same group by showing that deficiency of DAF significantly enhanced T lymphocyte responses to active immunization, and exacerbated the T-lymphocyte–dependent experimental autoimmune encephalomyelitis model.[59]

Similar to human CR1, Crry is an intrinsic membrane complement inhibitor that inhibits C3 convertases of all pathways through decay-accelerating and factor I cofactor activities for C3b and C4b, combining activities of human DAF and membrane cofactor protein.[60] Crry is expressed widely in most mouse tissues, particularly at potential sites of IC deposition and damage, such as the mesangium and arterial endothelium.[61] The role for Crry in limiting complement activation in these sites is supported by a series of insightful studies from the Matsuo and Okada laboratories.[62-65] Given that the Crry-deficient mice generated by Molina et al have complete embryonic lethality from unrestricted maternal complement activation,[66] the exact role for Crry in murine SLE and lupus nephritis awaits the generation of Crry-deficient lupus mice, which requires fairly elaborate experimental strategies to surmount this embryonic lethality. That Crry will be relevant in lupus nephritis seems almost inescapable based on what we know already. Additional support for this comes from our recent studies of chronic serum sickness in $Crry^{-/-}C3^{+/-}$ mice and transplantation of $Crry^{-/-}C3^{-/-}$ kidneys into wild-type hosts. In the former, the glomerular disease phenotype was worsened compared with controls, whereas in the latter there was marked inflammation in the tubulointerstitium that led to complete failure of the transplanted kidney within weeks (although the appropriate controls, including wild-type kidneys transplanted into wild-type or $Crry^{-/-}C3^{-/-}$ mice, remained normal).

Soluble CR1 is a recombinant form of human CR1 lacking its transmembrane region and short cytoplasmic tail. Similar to native CR1, soluble CR1 inhibits complement activation by accelerating the decay of both alternative and classic pathway C3/C5 convertases, and by acting as a factor I cofactor for the cleavage and inactivation of C3b. That inhibiting complement activation at the C3/C5 convertase step would be worthwhile in lupus nephritis was suggested by studies performed by Couser et al[67] in 3 rat models of glomerular diseases that collectively had manifestations comparable with lupus nephritis. Transgenic mice developed by our group that overexpressed a soluble form of Crry had inhibited complement activation systemically and locally in the kidney.[68] When crossed into the MRL/*lpr* strain, Crry complement inhibited MRL/*lpr* mice had lessened lupus nephritis as shown by lower blood urea nitrogen and urinary albumin values. Because the spontaneous mortality in lupus mice is largely caused by kidney disease, this translated into prolonged survival, whereas the underlying abnormal autoimmunity was not affected.[69] To make this complement inhibition more applicable to human SLE treatment, a recombinant soluble form of Crry fused to the hinge CH2 and CH3 domains of mouse IgG1 (Crry-Ig),[70] also was used in MRL/*lpr* mice. In chronic use from early in the autoimmune disease until the end stage, the inhibited complement activation by Crry–Ig ameliorated lupus nephritis.[71] Interestingly, transcript profiling experiments showed that excessive matrix components such as collagens I, III, IV, and VI were overexpressed in control MRL/*lpr* mice compared with MRL/$Mp^{+/+}$ strain controls, which was suppressed by complement inhibition by Crry–Ig. Potential explanations for these phenomena were the Crry-Ig–mediated reductions in connective tissue growth factor and transforming growth factor-β1 expression, suggesting these profibrotic agents may contribute to the progressive glomerulosclerosis in MRL/*lpr* mice in a complement-dependent manner.[72]

CR1 and CR2 are glycoproteins encoded by separate genes in human beings, whereas in mice they are the alternatively spliced product of a single *Cr2* gene.[73] Therefore, knockouts of the mouse *Cr2* gene leads to CR1 and CR2 protein deficiencies. In both human and experimental SLE, CR1/CR2 expression decreases, suggesting a relevance or at least involvement

with disease.[74,75] In C57BL/6$^{lpr/lpr}$ mice, deficiencies of C4 or CR1/CR2 result in autoimmune disease,[76,77] suggesting that classic pathway activation on antigenic material (such as derived from apoptotic debris in lupus) can maintain tolerance via B-lymphocyte CR1/CR2 signaling.[78,79] Yet, MRL/*lpr* mice deficient in CR1/CR2 had significantly lower levels of total IgG3 and specific IgG3 rheumatoid factor, supporting the role of CR1/CR2 in production of IgG3 in response to autoantigens. Nonetheless, this decrease of IgG3 autoantibodies did not lead to a reduction in the features of lupus nephritis.[80]

In physiologic situations, there is spontaneous, continuous, low-level alternative pathway activation that is restrained by effective complement regulation. Observational studies in human beings, and more recently in animals with spontaneous or targeted CFH deficiencies, have clearly illustrated the primary role of CFH as the alternative pathway regulator,[81-83] yet even fully functional CFH can be overwhelmed by the tempo of complement activation. Although the traditional thinking is that SLE is induced through IC-directed classic pathway activation without involvement of the alternative pathway, Gilkeson et al showed that CFB- or CFD-deficient MRL/*lpr* mice had reduced glomerular C3 deposition associated with less severe glomerular histopathology.[84,85] These results imply that IC-directed classic pathway activation can recruit the potent alternative pathway to further amplify generation of C3 and C5 activation products.

Compared with extensive studies focusing on C3 activation and its regulation, fewer studies have been performed to investigate the downstream events after C3 activation in the pathogenesis of SLE. The important study of Wang et al[86] used a specific function-inhibitory monoclonal antibody to C5 in NZB/W mice. Six months of continuous therapy led to significantly delayed onset of proteinuria, improved renal pathologic changes, and prolonged survival, implicating products of the terminal complement pathway, namely C5a and C5b-9, in lupus nephritis.

Anaphylatoxins C3a and C5a are generated through complement activation when C3 and C5 are activated and cleaved. By signaling through C3aR and C5aR, they play a role in leukocyte accumulation occurring in various inflammatory diseases, including GN.[26] C3aR and C5aR expression was upregulated significantly at both the messenger RNA and protein levels and accompanied by a wider cellular distribution in MRL/*lpr* mouse kidneys.[8,87] This upregulated expression started before the onset of kidney disease, supporting C3aR and C5aR may be involved in the development of disease, rather than simply a consequence. Chronic administration of a specific C3aR antagonist led to significantly reduced kidney disease and prolonged survival in MRL/*lpr* mice.[8] Similarly, when C5a signaling was blocked in our studies with a specific antagonist[87] or in those by Braun's group through gene targeting,[88] MRL/*lpr* mice animals displayed attenuated renal disease and prolonged viability. The effects of blocking C3aR and C5aR in lupus mice had certain features in common, including a reduction in renal neutrophil and macrophage infiltration, apoptosis, and interleukin-1β expression.[8,87] Effects on chemokine expression were distinct, with C3aR- and C5aR-inhibited MRL/*lpr* mice having reduced CCL5 (regulated upon activation, normal T cell expressed and secreted [RANTES]) and CXCL2 (macrophage inflammatory protein [MIP]-2) expression, respectively. C3aR-inhibited mice also had increased phosphorylation of protein kinase B (Akt), which we considered suggestive that C3aR signals promote renal cell apoptosis through an Akt pathway.[8] In C5aR-deficient MRL/*lpr* mice, there was a reduction in CD4$^+$ T-cell renal infiltration, lower titers of anti–double-stranded DNA antibodies, and inhibition of interleukin-12 p20 and interferon-γ production, supporting that Th1 responses are important to link C5a signaling in lupus nephritis.[88]

As discussed previously, C3 is the converging point for all 3 complement pathways. Alterations in C3 activation through manipulating its regulators, such as Crry or DAF, or blockade of the effects of C3 activation with inhibitors of C3aR, C5aR, and C5, have shown that C3 activation is an important factor in the development of SLE. Surprisingly, C3 deficiency did not affect the development of nephritis in MRL/*lpr*

mice, whereas glomerular IgG deposition was increased significantly.[89] This study is consistent with the important role of complement, and in particular C3, in the clearance of ICs.[32,90] The most likely explanation for why there still was inflammatory glomerular disease is that excessive IC deposition resulted in a greater degree of cellular accumulation via Fcγ receptors (FcγRs). The relative roles of complement activation products and their receptors and FcγRs remains an unsettled area in lupus nephritis.[91,92]

As discussed before, the complement system is an important part of innate immunity, which provides a number of benefits, such as imparting resistance toward infectious agents, processing of naturally generated ICs and apoptotic material,[27,30] and the development of an optimal and directed humoral immune response.[93,94] In theory, each of these may be affected with chronic systemic complement inhibition, which is supported by some experimental evidence.[71,95] The alternative approach of selectively targeting the desired complement regulator to the site of tissue injury has been advanced largely through the work of the Tomlinson group first in proof-of-principle experiments[96,97] and more recently in disease states.[95,98,99] Relevant to proteinuric renal diseases in general is that Crry and CD59 targeted to the rat proximal tubule using a monoclonal antibody approach protected rats from tubular injury in puromycin-induced proteinuria.[98] Given that sites of complement activation in the glomerulus and elsewhere are marked by the presence of C3d,[100] this represents an ideal target for therapy, as can be accomplished with CR2, given its natural affinity for C3d. Thus, chimeric CR2-Crry is targeted to the glomerulus in lupus nephritis,[99] where it can exert a therapeutic effect (Tomlinson S, unpublished data).

WHAT WE HAVE LEARNED FROM ANIMALS CAN BE USED IN THE TREATMENT OF HUMAN BEINGS

Strategies to manipulate the complement system in different human diseases have followed from successful animal studies, including through the use of recombinant intrinsic complement regulators and blocking antibodies. In addition to the treatment approaches indicated in studies using experimental models as we discussed earlier, we focus on therapeutic approaches that have been used in the treatment of human diseases, which potentially may extend to the treatment of human SLE and lupus nephritis.

Soluble CR1 (designated TP10, Avant Immunotherapeutics, Needham, MA) has been used in clinical trials in several human diseases. In a phase I clinical trial in patients with acute respiratory distress syndrome, the half-life of TP10 was 33.4 to 94.5 hours, with a dose-dependent reduction in CH50 values.[101] More recently, 2 separate, randomized, multicenter, placebo-controlled, double-blind, phase II clinical trials showed protective effects of TP10 in ischemia-reperfusion injury in patients undergoing lung transplantation or cardiac surgery. The first one involved 33 medical centers in the United States. A total of 564 high-risk patients undergoing cardiac surgery received an intravenous bolus of sCR1 (at 1, 3, 5, or 10 mg/kg) or placebo immediately before cardiopulmonary bypass. TP10 reduced the incidence of death or myocardial infarction in males by 36%, although the beneficial effect did not extend to female patients.[102] The other clinical trial was conducted at 4 North American lung transplant centers in which a total of 59 patients were given a single 10 mg/kg TP10 dose intravenously. Of the variables examined, TP10 significantly reduced the number of patients who were ventilator-dependent at 24 hours (50% versus 81%), indicating TP10 decreases the severity of ischemia-reperfusion injury in patients after lung transplantation.[103]

The most extensively used antibody targeting the complement system is a monoclonal antibody that directly binds human C5 and prevents its cleavage to C5a and C5b, either as a humanized monoclonal antibody (Eculizumab, Alexion Pharmaceuticals, Inc., Cheshire, CT) or its single-chain variable fragment (ScFv) form (Pexelizumab, Alexion Pharmaceuticals, Inc., Cheshire, CT). The latter has had some efficacy in patients undergoing cardiopulmonary bypass and coronary artery bypass grafting.[104,105] Paroxysmal nocturnal hemoglobinuria is an acquired disorder of glycosylphosphatidylinositol-

linked proteins including DAF and CD59, characterized by spontaneous complement activation and C5b-9–mediated hemolysis.[12] Based on its known pathophysiology, it is gratifying that Eculizumab has been shown to be effective therapy for this condition,[106] which lends itself to long-term use.[107] Moving to the kidney, idiopathic membranous nephropathy is also a disease in which C5b-9–mediated effects (in this case on the podocyte) are predicted to be pathogenic from a large amount of animal data.[108] A multicenter phase II trial in the United States in which 122 patients with idiopathic membranous nephropathy were enrolled in a randomized, placebo-controlled study of Eculizumab has been completed. Unfortunately, there was no difference comparing treatment with placebo in the primary outcome variable of urinary protein excretion. Because of the short-term treatment strategy in a long-term disease, the study design may have been insufficient to uncover a true therapeutic effect. This is supported by the finding of an apparent benefit in patients enrolled in an open-label extension. As noted earlier, long-term treatment with anti-C5 had impressive effects on lupus nephritis in NZB/W lupus mice.[86] Given this as well as favorable phase I safety data in patients with SLE, we designed a multicenter phase II trial using Eculizumab in proliferative lupus nephritis that was even backed by the US National Institutes of Health. Unfortunately, after enrollment of our first patient, this trial encountered logistic delays and ultimately came to a complete halt. This is not an indictment of a lack of real or potential efficacy, rather it reflects the difficulties in clinical trials for a disorder such as lupus nephritis.

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