Is complement a target for therapy in renal disease?

PETER W. MATHIESON

Academic Renal Unit, University of Bristol, Bristol, England, United Kingdom

Is complement a target for therapy in renal disease? Complement deposition in the injured kidney is common, especially in glomerulonephritis. The precise role of the complement system in the mediation of tissue injury in the kidney has been defined in recent years, and this has assumed extra importance with the recent development of specific forms of therapy directed at the complement pathway. As well as the induction of cell lysis, complement has many subtle effects on cell biology, particularly on endothelial cells. Complement components are produced locally in the kidney. Detailed studies of certain rare forms of nephritis have provided evidence that complement activation can directly cause tissue injury. Appreciation of the importance of complement in hyperacute rejection of xenotransplants has given new impetus to the development of complement inhibitors. A narrative review is provided, with a brief overview of the complement pathway and its regulatory mechanisms, mechanisms of complement-induced tissue injury, local complement production, and the renal consequences of complement dysregulation. Currently available forms of therapy aimed at the complement system are reviewed, and possible future therapeutic strategies are suggested. The complement system plays a direct causal role in tissue injury in certain forms of renal disease. Specific forms of therapy are becoming available that can selectively interrupt complement activation or promote its regulation. Much of the drive for the development of these therapies comes from the field of xenotransplantation, but these forms of therapy should also be tested in various primary renal diseases.

The complement pathway is a phylogenetically ancient system of interacting proteins that forms part of the host defence response. Complement has been of interest to nephrologists and renal pathologists ever since techniques of immunohistological analysis allowed the demonstration of complement components deposited in the kidney in certain forms of glomerulonephritis, associated in some cases with evidence of systemic complement activation. However, whether such evidence of local or systemic complement activation is causally related to tissue injury in the kidney, or merely an epiphenomenon, has remained controversial. This argument remained only of academic rather than clinical significance while there were no specific methods of achieving selective blockade of complement activation. Several recent advances have suggested that this is an area that deserves further attention from those interested in the pathogenesis and treatment of kidney disease. First, it has become apparent that complement is not only involved in the lysis of target cells. There are many more subtle effects on cell biology that can result from sublytic complement attack or as a response to the by-products of complement activation. Second, there has been an increasing appreciation that complement components are produced locally in a variety of tissues, and that this is particularly prominent in the kidney. Third, detailed studies of certain rare forms of nephritis have indicated that complement activation may play a direct causal role in tissue injury. Fourth, and perhaps most significant, is the recent development of novel therapeutic approaches aimed at preventing or limiting complement activation systemically or locally. The purpose of this article is to review some of these recent advances and attempt to assess their significance for nephrology.

THE COMPLEMENT PATHWAY

Over thirty proteins are involved in the three pathways of complement activation (Fig. 1), and many of these, some cell-bound and some secreted, have regulatory functions that impose tight control on complement activation. This complex regulatory machinery prevents unnecessary or excessive complement activation in health. The classical pathway is mainly activated by antigen/antibody complexes. This is the pathway responsible for the augmentation of the effector functions of antibodies and also is concerned with the effective handling of immune complexes by maintenance of their solubility, allowing their delivery to the reticulo-endothelial system for safe disposal. The alternative pathway is more concerned with host defence, being mainly activated by foreign surfaces such as microorganisms. It has two distinguishing features: activation is antibody-independent, and the pathway has a constant low-level of activation, existing in a state of so-called “tickover.” Therefore, regulation is critical to prevent excessive activity, and an understanding of this regulatory mechanism is crucial to the explanation of the dysregulated alternative pathway.
pathway activation associated with some forms of renal disease, to be considered later. Regulatory mechanisms exist in the fluid phase and on the cell membrane, and these two groups of inhibitors work together to downregulate inappropriate complement activation. The rate-limiting enzyme is the alternative pathway C3 convertase, denoted C3bBb. This enzyme is unstable, having a half-life of only a few minutes \(\text{in vivo}\). Its dissociation is promoted in the fluid phase by two regulatory proteins factor H and factor I, and on the cell membrane by decay accelerating factor (DAF). A recently described third pathway of complement activation is the lectin or mannan-binding protein pathway \[1\], which has similar consequences to those of classical pathway activation. The relevance of this pathway to renal disease is not yet known.

MECHANISMS OF COMPLEMENT-INDUCED INJURY

Activation of either the classical or alternative pathway leads to a final common pathway of complement activation, the terminal pathway. This culminates in the formation of the membrane attack complex (MAC or C5b-9), which when inserted in sufficient quantity into the plasma membrane of target cells leads to the formation of pores, entry of water and extracellular ions, cell swelling, and ultimately cell lysis. It has become apparent in recent years that C5b-9 also has other potent effects on the biology of target cells, and that sublytic complement activation may be an important phenomenon \(\text{in vivo}\). Much of the work on this subject relates to endothelial cells, but other intrinsic renal cells such as mesangial cells or podocytes may be affected in a similar way. Production of C5b-9 on the endothelial cell surface has procoagulant and pro-inflammatory consequences, leading to rapid influx of calcium into the cell, secretion of Weibel-Palade bodies with release of procoagulant high molecular weight forms of von Willebrand factor, exposure of binding sites for factor Va, release of platelet activating factor that stimulates the adhesion and aggregation of platelets, and upregulation of the expression of GMP-140, a \(\alpha\)-selectin that promotes the adherence of neutrophils \[2\]. By-products of complement activation may further amplify the process. For example, C3a and C5a, by-products respectively of cleavage of C3 and C5 (by either pathway), act as anaphylatoxins that attract and
activate leukocytes, recruiting them to the inflammatory focus. C5a also leads to the release of procoagulant heparan sulphate from endothelial cells [3]; deposition of iC3b, a ligand for the complement receptor CR3 on neutrophils, on the endothelial cell surface will further promote the accumulation of inflammatory leukocytes [4]. Complement activation also has effects on platelets, with calcium-dependent activation of protein kinases leading to secretion of procoagulant molecules from storage granules [5]. Thus, the net result of complement activation on platelet-endothelial interactions is a shift towards a more procoagulant state. Sublytic C5b-9 also has potent pro-inflammatory effects on mesangial cells in vitro [6, 7]. The recent demonstration that human mesangial cells have receptors for the anaphylotoxin C5a, and that binding of C5a to mesangial cells induces upregulation of transcription factors and early response genes [8], illustrates a novel mechanism whereby complement activation may directly influence resident cells in the kidney, in addition to the pro-inflammatory effects mediated via leukocyte attraction.

It has been known for many years that complement influences the afferent arm of an immune response. Complement-depleted mice are less able to mount an antibody response [9]. A mechanism for this is illustrated by recent work showing that C3d, a breakdown product of C3 and the ligand for complement receptor CR2, can act as an adjuvant: antigen tagged with C3d molecules was much more potent at eliciting an antibody response, presumably due to cross-linking of CR2 molecules on B lymphocytes [10].

LOCAL COMPLEMENT PRODUCTION

Most complement components are produced in the liver, but local production in a number of organs is now well documented [11]. In the kidney this has been most clearly shown for C3 and C4, which are synthesized and secreted by renal tubular epithelial cells, mesangial cells and glomerular epithelial cells [12]. This expression is upregulated by pro-inflammatory cytokines, and also in various forms of nephritis. Factor B is expressed in normal kidney, and possibly also factor D [13, 14]. Complement regulatory molecules including decay accelerating factor (DAF, CD55) and membrane cofactor protein (MCP, CD46) are expressed in the kidney in health and disease [15, 16]. Another tissue capable of local production of complement components is the vascular endothelium. Endothelial cells lie at the interface between the tissues and the circulation, and complement activation on the endothelial surface is a common feature in many forms of tissue injury. As with other tissues, the relative contributions of locally produced complement and circulating complement remain to be determined. Certainly the local expression of regulators of complement activation makes teleological sense, since there may be a need to control complement activation at this site. Endothelial cells abundantly express membrane-bound regulators of complement activation such as CD59, MCP and DAF, and this underlies the strategy for prevention of hyperacute xenograft rejection by transgenic expression of the human versions of these regulators in pigs [reviewed in 17]. Factor H, important in regulation of the alternative pathway, is also expressed by endothelial cells. Intriguingly, pro-inflammatory cytokines (which enhance endothelial synthesis of C3 and factor B) lead to downregulation of factor H, tipping the balance in favor of activation, presumably as a defence mechanism [18]. Hemolytic-uremic syndrome (HUS) is characterized by marked endothelial injury, especially in the kidney, and it is therefore fascinating that defective complement regulation may predispose to this condition. This is considered further in the next section.

RENAL CONSEQUENCES OF COMPLEMENT DYSREGULATION

As mentioned earlier, tight regulatory mechanisms exist to limit complement activation in health. For the alternative pathway, factor H is a key regulatory protein, and the consequences of loss of its regulatory effects are amply illustrated by study of individuals whose ability to produce this protein is deficient. This has been best studied in a variety of Yorkshire pig [19], but rare human cases have also been reported [20]. In the pig, genetic factor H deficiency leads to the development of glomerulonephritis (GN), with morphological appearances directly analogous to mesangiocapillary GN type II in humans (also known as membranoproliferative GN type II or dense deposit disease), which leads to the rapid development of renal failure and death from uremia [19]. The pigs have evidence of unregulated systemic alternative pathway activation, and importantly it has recently been shown that administration of exogenous factor H is an effective therapy, even if treatment is not started until after GN is established [21]. These animals prove that alternative pathway dysregulation alone can cause nephritis, and that restoration of the capacity to regulate the alternative pathway is sufficient to restore health. In humans, genetic factor H deficiency may also lead to MCGN [20], and the molecular basis of the defect has recently been characterized in one such patient. Mutations leading to loss of cysteine residues altered the protein structure in such a way that intracellular processing of factor H was disrupted [22]. The importance of functional factor H is further illustrated by a case report of an individual whose serum contained a monoclonal lambda light chain that interacted with factor H in vitro and prevented its action, allowing unregulated alternative pathway activation. That patient also developed type II MCGN [23]. Thus, disruption of the action of factor H by whatever mechanism can lead to MCGN. Another area of recent research should be mentioned that concerns HUS, a condition of great interest to nephrologists. One early report indicated that factor H deficiency might predispose to HUS [24], and certainly complement activation is conspicuous in
this condition [25]. Rarely, recurrent HUS occurs in a familial form, and it has recently been reported that mutations of the factor H gene in these families are associated with unregulated complement activation and episodic HUS [26]. The relevance of this to more common forms of HUS is not yet certain, but it is noteworthy that an abnormality of the factor H gene has been found in at least one individual with sporadic HUS [26] and that the factor H gene is polymorphic [27, 28], leading to the possibility that genetic variability at this locus could be associated with susceptibility to disease.

Another situation in humans where unregulated alternative pathway activation is associated with MCGN is in the presence of nephritic factor (NeF), an IgG autoantibody that binds to a neoantigen formed when the alternative pathway C3 convertase enzyme, C3bBb, is assembled. The antibody stabilizes this enzyme and protects it from degradation by factor H. Thus, the half-life of the enzyme is prolonged and the normal regulatory mechanism is subverted. The close association between NeF and MCGN implies, but does not prove, causality [29]. However, the fact that similar renal injury arises when the alternative pathway is dysregulated by the other mechanisms outlined above, namely factor H deficiency or dysfunction, strongly implies that it is the complement activation per se which induces the nephritis. The other clinical condition associated with NeF, with or without coexisting nephritis, is partial lipodystrophy (PLD) in which there is loss of adipose tissue from the face, arms and upper trunk [30]. Adipocytes are another abundant source of local complement production, particularly of factor D, which plays a central role in normal adipose tissue regulation [reviewed in 31]. We have reported that NeF-containing serum or IgG, but not normal or disease-control sera/IgG, could induce complement-dependent lysis of adipocytes in vitro [32, 33]. Furthermore, there are regional variations in levels of expression of factor D that mirror the distribution of fat cell loss in PLD, the higher levels being found in fat from the face and upper trunk [33]. Thus, NeF appears capable of directly causing adipocyte destruction, and the level of production of factor D by adipocytes may determine their susceptibility to injury by NeF. Since intrinsic renal cells express an array of complement proteins similar to those expressed by adipocytes, the obvious implication is that NeF may directly injure renal cells in an analogous manner. As yet there is no direct evidence for this action, but a recent report showed that alternative pathway activation can injure renal tubular epithelial cells [34]. Lupus nephritis is the archetypal immune complex nephritis, where complement activation in the glomerulus is assumed to be important, and therapy aimed at complement has been advocated. Intriguingly, a very recent report in a murine model of lupus strongly suggests that Fc receptor-mediated mechanisms of inflammation are more important that those involving complement [35]. The importance of complement in renal disease is not, however, confined to rare esoteric forms of glomerulonephritis as complement activation may play an important role in other, more common forms of renal injury. For example, there is a wealth of evidence for a vital role of complement in ischemia-reperfusion injury. This is important to nephrologists in the context of transplantation, after revascularization for renovascular disease, and possibly also in some forms of ischemic acute renal failure. Complement inhibition provides substantial protection against this form of injury [36–38], probably by inhibiting leukocyte attraction and infiltration [38]. Another important and possibly underestimated cause of renal injury is embolization of material from atheromatous plaques [39]. One feature of the syndrome associated with cholesterol emboli is systemic complement activation leading to hypocomplementemia [40], and it has been shown that components of atheroma are potent activators of complement [41, 42]. The therapeutic potential of complement inhibition in patients suspected of having this form of renal injury may be considerable but as yet remain unexplored. Indeed, complement may be involved in atherosclerosis itself. The inflammatory nature of atherosclerotic vascular disease is increasingly being appreciated. In one experimental model, deposition of C5b-9 was one of the earliest histological manifestations, preceding monocyte infiltration and foam cell development [43], indicating that the terminal complement pathway is likely to be involved in atheroma development and that measures aimed at complement inhibition may have therapeutic potential in atherosclerosis. There may be implications for the role of complement in chronic allograft rejection, elements of which have similarities to atherosclerosis [44]. Another example of the role of the complement system in microvascular injury in vivo is illustrated by experiments examining renal injury in mice rendered hypertensive by uninephrectomy and treatment with desoxycorticosterone. Mice with C5 deficiency, unable to assemble C5b-9 after proximal complement activation, showed a dramatic reduction in glomerular damage compared to congenic mice with an intact terminal complement pathway [45].

**THERAPY AIMED AT COMPLEMENT**

Current anti-inflammatory and immunosuppressive treatments are nonspecific. It is quite possible that corticosteroids, for example, exert some of their effects by interference with complement-mediated injury. As we aim for more selective forms of intervention, the precise identification of targets will become ever more important. Appreciation of the role of complement in renal injury has accumulated over the years, but only recently have specific methods of complement blockade become available.

It is beyond the scope of this article to consider the strategies being used to overcome complement activation in xenograft rejection, or indeed in transplantation in general. These have been recently reviewed elsewhere [17].
and will only be considered here when they shed light on possible therapeutic approaches for native renal disease. Until recent years, studies of the role of complement in tissue injury have relied on rare genetic variants where individual complement components are deficient, or on crude methods of complement depletion such as the use of cobra venom factor. “Accidents of nature” with complement deficiency have given useful information about the importance of complement in induction of tissue injury. For example, in an experimental model of membranous GN induced in rabbits with cationized bovine serum albumin, C6 deficient rabbits showed glomerular morphology and deposits of immunoglobulin and C3 that were identical to those seen in normal rabbits, but the C6 deficient animals did not develop proteinuria [46]. This may give hints about useful therapeutic targets for human membranous GN, that is, selective interference with C5b-9 may be possible with monoclonal antibodies to individual components. This therapeutic strategy has already been shown to be successful in passive Heymann nephritis in the rat [47] and in a murine model of lupus [48]. As yet there is no experience with this form of therapy in human disease. Studies of complement-deficient individuals have also illustrated potential hazards of therapy with individual complement components. For example, in C3 deficient dogs (which develop MCGN) treatment with exogenous C3 in the form of normal dog serum was associated with a worsening of the nephritis [49]. Similarly, C6-deficient humans, who are at increased risk of Neisserial infections, may be actively harmed by plasma therapy due to enhanced complement activation when an intact pathway is restored by provision of a fresh supply of the missing protein [50].

With the advent of gene knockout technology, further genetic mutants are available to address the role of individual complement components [51], and these animals will provide a useful resource for experimental studies, helping to elucidate the relative importance of each pathway of complement activation in health and disease. One particularly striking example is the recent report that mice with targeted disruption of the C1q gene, which are completely deficient in C1q and therefore unable to achieve any activation of the classical complement pathway, develop a lupus-like nephritis [52]. This in itself may not be particularly surprising since C1q deficiency in humans is associated with severe lupus [53]. However, the intriguing observation in the C1q knockout mice was the associated increase in apoptotic bodies in their glomeruli, irrespective of the severity of glomerulonephritis [52]. This implies that C1q, and hence the classical complement pathway, plays a role in the normal clearance of apoptotic cells from the glomerulus; a similar pathway has recently been proposed for the clearance of apoptotic keratinocytes [54]. The provocative interpretation of these results is that, since the typical autoantigens in lupus are nuclear or cytoplasmic components that are normally sequestered intracellularly and therefore not accessible to the immune system, C1q deficiency may predispose to autoimmunity by allowing persistence of apoptotic cells that display autoantigens on their surface [52, 54]. Enhancement of clearance of apoptotic cells by promotion of this function of the classical pathway could provide a novel therapeutic strategy for lupus or other autoimmune nephritides.

One major problem with cobra venom factor (CVF) is that in order to bring about complement depletion, it first causes massive complement activation. Thus, any beneficial effects of complement depletion may be masked by the results of this initial activation phase [55]. Furthermore, CVF is highly immunogenic, leading to the generation of a neutralizing antibody response so that CVF therapy becomes ineffective within 5 to 10 days. Efforts are being made to produce less immunogenic variants that retain decimating activity.

In designing therapies to regulate complement activation, it seems logical to exploit the fact that the complement system includes a number of naturally-occurring regulators. One example was alluded to earlier, that is, administration of purified factor H to animals in which this protein was deficient led to correction of their complement regulatory defect [21]. Whether there would be any value in administration of supraphysiological doses of factor H to individuals who are not factor H deficient, but have excessive alternative pathway complement activation for some other reason, remains unknown. Another naturally occurring complement regulator is complement receptor type 1 or CR1 (also known as CD35) [56]. This is a membrane-bound molecule that binds C3b and C4b and is normally expressed on erythrocytes, myelomonocytic cells, B lymphocytes, some T lymphocytes, and on glomerular epithelial cells. CR1 dissociates both classical and alternative pathway C3 convertases, and is therefore a potent inhibitor of complement activation via both pathways. There is a naturally occurring soluble form (soluble CR1, sCR1) that is present in tiny concentrations in plasma [57]. The role of this molecule in normal regulation of complement in the fluid phase in vivo is unknown, but its potency as a complement inhibitor made this a logical choice for development as a therapeutic agent. Recombinant sCR1 is now available and has been shown to have potent effects on tissue injury in models of ischemia-reperfusion [36–38], xenotransplantation [58, 59] and allotransplantation [60]. There is evidence from one xenograft model that an agent inhibiting neutrophil adhesion to endothelium by preventing up-regulation of the CD11b/CD18 adhesion molecule has synergistic effects with sCR1 [61], illustrating the point that complement inhibition may only form one component of novel therapeutic approaches. Promising results with sCR1 have been reported in three experimental models of renal disease: passive Heymann nephritis, antithymocyte serum-induced mesangial proliferative GN, and concanavalin A-induced diffuse proliferative GN [62]. Each of these models
is known to be complement-dependent, and in each case sCR1 reduced proteinuria and improved indices of glomerular inflammation. However, very large doses of sCR1 were used (60 mg/kg/day), and the logistic and economic implications of therapy at similar dosages would be considerable for human therapy. Furthermore, the relevance of these observations to human GN is limited by the fact that the effects were seen with sCR1 pre-treatment, that is, before the induction of disease. It remains to be seen whether similar benefits are seen when treatment is delayed until after disease has become established, more closely resembling the clinical situation.

There is a brief report of the effectiveness of a compound called naftamostat mesilate on immune complex GN in humans (3 patients with lupus nephritis and 2 with cryoglobulinemia), together with the claim that this serine protease inhibitor exerts its effects by blockade of classical pathway complement activation [63]. There are many other possible explanations of the reduction of proteinuria reported with this agent, and these results await confirmation. Nevertheless, the general principle that therapy aimed at the complement system can have useful benefits is supported by this kind of clinical experiment. A semisynthetic polysaccharide called pentosan polysulfate, a potent inhibitor of complement activation in vivo, has recently been shown to have useful effects in a rabbit model of ischemia-reperfusion injury [64]. This agent has not yet been tested in any models of nephritis or in human disease.

**FUTURE APPROACHES**

Administration of other exogenous complement inhibitors has been studied recently, mainly in the context of xenotransplantation. Of particular interest are those molecules, including CD59 and DAF, which are linked to the cell membrane via a glycosylphosphatidylinositol (GPI) anchor. A valuable property of such molecules is that when present in a soluble form they can reinsert into cell membranes, raising the possibility that infused proteins could become incorporated into the membranes of target cells. Porcine endothelial cells can be protected from lysis by human serum by the administration of exogenous CD59 or DAF [65]. Thus, it would be theoretically possible to protect an organ from complement-mediated attack by infusing complement regulators locally. The usefulness of such an approach may be limited by the fact that serum lipoproteins inhibit the incorporation of GPI anchors into cell membranes [66], and also because rapid membrane turnover would mean that treatment would need to be repeated at frequent intervals. The problems of exogenous administration of complement regulators would be avoided if transgenic technology could be used to allow local expression of these molecules at the site of injury. Again, this approach so far mainly been proposed for xenotransplants, but there is no reason why similar techniques could not be exploited in native organs. In vitro, transfection of a complement regulator provides potent protection to mesangial cells against complement-mediated injury, either lytic or sublytic [67]. The main limitations to such gene therapy approaches at present concern the lack of suitable vectors for delivery of genes to the target organs or cells in vivo.

As mentioned earlier, individual complement components can be targeted specifically, for example, by monoclonal antibodies that block their activity. Prevention of the assembly of C5b-9 seems most likely to have therapeutic value, and using anti-C6 antibodies in this way is beneficial in passive Heymann nephritis in the rat [47]. However, the importance of C5b-9 in the active form of this condition, which more closely mimics human membranous GN, is disputed [68] and it is therefore not certain that a similar approach would be useful in the analogous human condition. It may also be worth testing this type of therapy in other forms of GN. There is some evidence for the role of C5b-9 in mesangial proliferative GN [69] and in murine lupus [48]. The C5a receptor on leukocytes is a powerful mediator of inflammation because the blockade of this molecule by antibodies or by specific antagonists has powerful effects on leukocyte recruitment and activation. One potent group of C5a receptor antagonists, generated by modification of the C terminus of C5a, has recently been reported [70], and these agents showed potent inhibitory effects on neutrophil chemotaxis, adherence and activation in vitro together with promising anti-inflammatory effects in vivo in rabbits and pigs. This type of anti-inflammatory therapy could have very wide applicability in human disease.

**CONCLUSIONS**

Complement plays a role in diverse forms of renal injury. Modern molecular biological techniques have opened new possibilities for selective immunotherapy, and interference with complement activation is no exception. In particular, the recognition of the importance of complement in hyperacute xenograft rejection has led to a resurgence of interest in the development of complement inhibitors. These advances may benefit patients with various forms of primary renal disease, possibly contributing to the field of transplantation in an unforeseen way: better treatment of primary renal disease leads to a reduced need for renal replacement!

Reprint requests to Professor Peter Mathieson, Academic Renal Unit, Southmead Hospital, Bristol BS10 5NB, England, United Kingdom.
E-mail: p.mathieson@bris.ac.uk

**REFERENCES**


