Translational Mini-Review Series on Complement Factor H: Therapies of renal diseases associated with complement factor H abnormalities: atypical haemolytic uraemic syndrome and membranoproliferative glomerulonephritis

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Summary
Genetic and acquired abnormalities in complement factor H (CFH) have been associated with two different human renal diseases: haemolytic uraemic syndrome and membrano proliferative glomerulonephritis. The new genetic and pathogenetic findings in these diseases and their clinical implications for the management and cure of patients are reviewed in this paper.

Keywords: alternative pathway of complement, complement factor H, haemolytic uraemic syndrome, membranoproliferative glomerulonephritis, nephritic factors

Introduction
Since the late 1990s, a growing number of reports has contributed to identify genetic and acquired abnormalities in complement factor H (CFH) and other regulatory components of the alternative pathway of complement that are associated with different human diseases. These include diseases of the kidney, the atypical form of haemolytic uraemic syndrome (aHUS) and membranoproliferative glomerulonephritis (MPGN), and of the eye, age-related macular degeneration (AMD). New findings on the pathogenetic mechanisms underlying these distinct diseases have provided crucial information on the role of regulation of the alternative pathway of complement in kidney and eye physiology and pathophysiology. The clinical implications of the new knowledge for prediction of clinical outcome and designing of effective and tailored therapies are the objective of this review, with the main focus on aHUS and MPGN (Table 1).

Haemolytic uraemic syndrome
Haemolytic uraemic syndrome is a disease of non-immune haemolytic anaemia, thrombocytopenia and renal impairment. The common microvascular lesion consists of vessel wall thickening with endothelial swelling and detachment [1]. In children, the disease is triggered most commonly by bacteria which produce Shiga-like toxins (Stx), certain Escherichia coli serotypes (O157:H7 is the most common) and Shigella dysenteriae serotype 1 [1,2]. This form of the disease usually has a good outcome, with complete recovery in about 80–90% of cases [1].

In approximately 10% of the HUS cases there is no evidence of an infection by Stx-producing bacteria. These atypical cases (aHUS) may occur sporadically or within families, are often recurrent and generally with a poor outcome [1]. Up to 50% of cases progress to end-stage renal diseases (ESRD) or have irreversible brain damage, and 25% may die during the acute phase of the disease [3–5].
Familial aHUS results from genetic abnormalities in circulating and membrane-bound proteins that regulate the complement system to prevent non-specific damage to host cells, and evidence is available that similar genetic alterations may also confer predisposition to sporadic aHUS [1]. In a few patients with sporadic aHUS, acquired immune abnormalities due to the formation of autoantibodies against CFH have been reported.

Identification of the specific abnormality underlying the disease could have important implications for more rational and tailored patient treatment and management [1] (Table 1).

### aHUS associated with genetic defects of complement regulatory proteins

More than 100 mutations in the gene encoding CFH, a plasma glycoprotein that controls both spontaneous activation of complement C3 in plasma and deposition of C3b on host cells, have been reported so far in patients with aHUS [6], which account for around 20–30% of cases [7,8]. Mutations in MCP, encoding membrane co-factor protein, a transmembrane complement regulator that inactivates C3b deposited on cell surface [9], and in CFI, encoding a plasma serine protease that cleaves and inactivates C3b and C4b, have been reported in 10–15% and 5–12% of patients, respectively [8,10–13]. More recently, two gain-of-function mutations in the gene encoding complement factor B (CFB), a zymogen that carries the catalytic site of the complement alternative pathway convertase, have been found in two families from a Spanish HUS cohort [14].

Finally, in rare published cases there is segregation of multiple genetic risk factors, such as combined mutations of either CFH and MCP or MCP and CFI, often associated with specific SNP haplotype blocks in either CFH or MCP [8,15].

The phenotype of aHUS patients is very variable; although many patients develop early-onset HUS and ESRD, others make a complete recovery [1]. Although genotype–phenotype correlations are not always very clear, identification of the specific genetic defect in a patient with HUS could enhance diagnostic precision and prediction of clinical outcome [1,7,8,16].

aHUS associated with CFH mutations often presents early in childhood, although adult onset is reported in around 30% of cases. The clinical course is characterized by a high rate of relapses and 60–80% of patients die or develop ESRD following the presenting episode or progress to ESRD as a consequence of relapse [8]. aHUS associated with MCP mutations presents mainly in childhood; the acute episode is, in general, milder than in CFH mutation carriers and 80% of patients undergo complete remission. Recurrences are very frequent but their effect on long-term outcome is mild, with around 60–70% of patients remaining dialysis-free even after several recurrences [8]. However, there are some exceptions, with a subgroup of patients who lost renal function either during the first episode or later in life. The clinical course of CFI mutated patients is more variable. Onset in childhood develops in half the patients. Fifty-eight per cent of patients develop long-term ESRD [8,12,13].

### Therapy

The mortality rate for aHUS dropped from 50% to 25% after plasma manipulation (plasma infusion or

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<th>Disease</th>
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<td>aHUS</td>
<td>CFH, CFI, CFB mutations Plasma infusion/exchange (often ineffective)</td>
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<tr>
<td>Genetic</td>
<td>MCP mutations Probably no indication to plasma infusion/exchange (relatively good outcome also with conservative therapy alone)</td>
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<td>Immune-mediated</td>
<td>Anti-CFH antibodies Plasma infusion/exchange combined with steroid or immunosuppressive therapy</td>
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<td>MPGN</td>
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<td>CFH mutations that do not block secretion Plasma infusion/exchange</td>
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<td>CFH mutations that block secretion Steroids (first line), other immunosuppressive therapy (second line) both combined with conservative and renoprotective therapies</td>
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<td>Immune-mediated</td>
<td>Nephritic factors (MPGN I and III) Plasma exchange (sometimes effective)</td>
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<td>CFH inhibitory antibody Plasma infusion/exchange combined with steroid or immunosuppressive therapy</td>
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CFH: complement factor H; CFI: complement factor I; CFB: complement factor B; MCP: membrane co-factor protein.
exchange) was introduced [17,18], and indeed a consistent number of patients respond to plasma treatment. It has been proposed that plasma exchange might be relatively more effective than plasma infusion as it might remove potentially toxic substances from the patient’s blood [19–21], and in one study plasma exchange was found to have superior efficacy to plasma infusion [22]. However, patients treated with plasma exchange were given larger amounts of plasma than those treated with plasma infusion alone and, when equivalent volumes of plasma were given, plasma infusion and exchange appeared to be equally effective [19]. In patients who are hypertensive and whose plasma volume is already expanded because of renal impairment, plasma exchange should be considered as the first-choice therapy [19]. In plasma exchange, one plasma volume (40 ml/kg) is usually exchanged per session. Treatment can be intensified by increasing the volume of plasma replaced to 100 ml/kg or more. If plasma exchange is not available, plasma infusion should be given: 30–40 ml/kg on day 1, followed by 10–20 ml/kg. Plasma treatment should be started within 24 h of presentation, as delay may increase treatment failure. Platelet count and serum lactate dehydrogenase (LDH) concentration are the most sensitive markers for monitoring the response to plasma therapy, which should be continued until they are persistently normalized. Discontinuation of plasma therapy is the only way to establish whether complete remission has been achieved, and many cycles of stopping and resuming plasma therapy may be required [1].

However, several patients do not respond to plasma infusions or become plasma-dependent and require long-term treatment, because the disease relapses when plasma infusion or exchange is stopped [19]. Up to 50% of patients with aHUS are exposed to large amounts of plasma in order to treat disease recurrences. In many cases, patients become sensitized to plasma components and are therefore exposed to acute hypersensitization reactions that may be life-threatening and require treatment with steroids and anti-histaminies.

Genotype–phenotype correlation studies have indicated that the genetic defect underlying the disease may influence response to plasma.

Plasma infusion or exchange has been used in patients with HUS and CFH mutations, with the rationale of providing the patients with normal CFH to correct the genetic deficiency. In published studies, some patients with CFH mutations did not respond at all to plasma and died or developed ESRD [8,23]. Others remained chronically ill or required infusion of plasma at weekly intervals in order to raise CFH plasma levels enough to maintain remission [24–27]. Stratton et al. [28] were able to induce sustained remission in a patient with a CFH mutation using 3 months’ weekly plasma exchange in conjunction with intravenous immunoglobulins. At 1 year after stopping plasma therapy, the patient remained disease-free and dialysis-independent. In our series [8], around 50% of patients with CFH mutations treated with plasma underwent either complete or partial remission (haematological normalization with renal sequelae). However, half the patients did not respond at all to plasma and 20% died during the acute episode. It is possible that the type and consequences of the CFH mutations influence response to either plasma infusion or exchange. Plasma infusion, by providing normal CFH, could be of benefit in those few patients with mutations that impair CFH secretion, causing complete or partial CFH lack in the blood, as suggested by complete recovery by fresh frozen plasma of a baby with undetectable CFH levels due to C1077W/Q1139X compound heterozygous mutations [25]. On the other hand, plasma exchange could be of more benefit in cases associated with mutations that result in a mutant CFH protein present in blood. In a recent report, in monozygotic twins carrying an heterozygous S1191L mutation that does not impair CFH secretion, plasma exchange showed benefit while plasma infusion alone did not [29]. It was hypothesized that mutant CFH in patient’s blood interferes with the function of the normal CFH, by forming non-functional dimers with the normal counterpart. In this setting plasma exchange could have the additional benefit of removing mutant CFH from patient circulation.

Because CFI and CFB are plasma proteins, plasma infusion and plasma exchange could theoretically be of value in patients with defects in the corresponding genes. Published data in a small number of patients document that about half of patients with either CFI [8] or CFB mutations [14] underwent remission following plasma infusion, exactly as observed in CFH mutated patients.

The rationale for using plasma in patients with membrane cofactor protein (MCP) mutations is not so clear, as MCP is a transmembrane protein and theoretically plasma infusion or exchange would not correct the MCP defect. Published data [8,11] indicate that the majority (70–80%) of patients underwent remission following plasma infusion or exchange; however, complete recovery from the acute episode was also observed in 70–80% of patients not treated with plasma. Due to the invasiveness of plasma therapy and the risk of sensitization that may limit the possibility of a successful transplant in those progressing to ESRD, it is probable that waiting before considering plasma therapy is the best strategy in cases associated with MCP mutations, as these forms appear to have a high rate of spontaneous remissions that do not appear to be increased appreciably by plasma therapy.

In occasional patients with extensive microvascular thrombosis at renal biopsy, refractory hypertension and signs of hypertensive encephalopathy, when conventional therapies including plasma manipulation are not enough to control the disease, bilateral nephrectomy has been performed with excellent follow-up [30]. The rationale of the procedure rests on evidence that removing the kidneys eliminates a major site of complement activation, which would limit platelet activation and protect patients from the further spreading of microvascular lesions. In a patient with
aHUS associated with a heterozygous MCP mutation, who was at imminent risk of death because of uncontrolled malignant hypertension, bilateral nephrectomy was followed within 2 weeks by complete haematological and clinical remission [8,30].

Transplantation. It is debatable whether kidney transplantation is appropriate for patients with aHUS who have progressed to ESRD. In published studies, around 50% of patients had a recurrence of the disease in the grafts [1]. There is no effective treatment of recurrences and graft failure occurs in more than 90% of such patients [31]. Patients who lost the first kidney for recurrence should not receive another transplant. Live-related renal transplant should also be avoided in that it carries the additional risk to precipitate disease onset in the healthy donor relative, as reported recently in two families [32].

Screening for mutations may help to define graft prognosis, and genotyping for CFH, MCP and CFI should be performed in all patients with ESRD secondary to aHUS being considered for transplantation. As CFH is a plasma protein produced mainly by the liver, a kidney transplant does not correct the CFH genetic defect. Indeed, in these patients the graft outcome is poor; the recurrence rate ranges from 50 to 100% according to different surveys, and is significantly higher than in patients without CFH mutations [1,7,33]. Intensive post-transplant plasma exchange has been attempted in a 12-year-old girl with a CFH mutation with the aim of preventing disease relapse on the graft [34]. However, 2 months after transplantation, concomitant with a reduction in plasma exchange frequency, renal function deteriorated and graft biopsy showed thrombotic microangiopathy with no sign of rejection. Despite daily plasmapheresis and replacement of cyclosporin with tacrolimus there was no improvement, and transplant nephrectomy was undertaken [34]. It is therefore very difficult to justify renal transplantation in patients with ESRD secondary to CFH-associated HUS.

Simultaneous kidney and liver transplant has been performed in two young children with aHUS and CFH mutations, with the objective of correcting the genetic defect and prevent disease recurrences [35,36]. However, both cases treated with this procedure were complicated by premature irreversible liver failure. The first case recovered after a second uneventful liver transplantation. The second case had a fatal, primary non-function of the liver graft followed by multi-organ failure and the patient’s death. The reasons for this may include increased susceptibility of the transplanted liver to ischaemic or immune injury related to uncontrolled complement activation. Additional combined kidney and liver transplants have been performed subsequently in three patients with CFH mutations [37,38], who received extensive plasma exchange pre- and post-transplant, in order to remove mutant CFH and provide enough normal CFH to prevent liver graft damage.

Of note in the latter two patients, heparin-based anticoagulation was started a few hours after the combined transplantation to prevent thrombotic events [38]. So far, all three patients who were treated with this regimen had a favourable post-transplant outcome with well-functioning grafts. Liver and kidney transplantation, combined with extensive plasma exchange (and heparin), may be an effective way to gain independence from chronic dialysis and may be life-saving in those infants who, on dialysis, have a poor life expectancy.

As CFI and CFB are plasma proteins, one could speculate that HUS recurrence may take place on the transplanted kidney, and patients may experience graft failure. The few data available are in line with this hypothesis, as graft failures for recurrence occurred in 15 of 18 patients with CFI mutations and in the only patient with CFB mutation who received a kidney transplant [8,14,39]. On the other hand, kidney graft outcome is favourable in patients with MCP mutations, as found in four patients who have been transplanted successfully with no disease recurrence [1,8,11]. There is a strong theoretical rationale for this: MCP is a transmembrane protein highly expressed in the kidney. Not surprisingly, transplantation of a kidney expressing normal MCP corrects the defects in these patients.

The picture is more complicated in patients with aHUS associated with multiple genetic hits. For them it is very difficult to predict graft outcome, as each genetic abnormality may have a different impact on the risk of relapses.

aHUS associated with anti-CFH antibodies

Recurrent, atypical HUS has been reported recently [40] in three children with circulating IgG autoantibodies against CFH. Anti-CFH antibodies were captured by enzyme-linked immunosorbent assay (ELISA) using purified human CFH coated plates. In a subsequent study, anti-CFH antibodies were identified in five unrelated children from a cohort of 60 patients. For these children, the binding site of CFH-autoantibodies was mapped and localized in the C-terminus of CFH [41]. Of interest, the children showed an increased titre of circulating anti-nuclear antibodies, a finding that supports the possibility of an autoimmune pathogenesis of the disease.

The prevalence of aHUS associated with anti-CFH antibodies ranges from 2 to 10% [40,41].

Therapy. Available information is insufficient to provide clear-cut guidelines to treatment of this rare form of HUS (Table 1). Conceivably, however, when anti-CFH autoantibodies are detected, plasma exchange and steroids, or other immunosuppressive agents, should be considered with the rationale of removing the pathogenic antibody from the circulation as soon as possible and inhibiting its synthesis. The plasma exchange procedure, in addition to supplying an extra amount of CFH that may saturate the autoantibody
activity, may also remove the autoantibody from the circulation.

Two children with anti-CFH antibodies recovered from the first episode with plasma exchange. They had four and three relapses, respectively, with heart involvement in one case that, again, recovered with plasma exchange [40]. These two children were maintained on chronic therapy with steroids or azathioprine, respectively. A third child received plasma infusions and intravenous immunoglobulin (IgG) infusion. He had two recurrences with pancreas and liver involvement, progressed to ESRD, and eventually required a bilateral nephrectomy to control refractory hypertension [40].

Steroids have been used extensively in the past to cure patients with atypical forms of HUS [19], with inconsistent results. In immune forms these treatments may help to inhibit the production of the anti-CFH autoantibody and, combined with plasma exchange, may result in an effective clearance of the autoantibody from the circulation. In contrast, they have absolutely no place in the treatment of genetic forms. Trials considering the two forms together invariably diluted the potential benefits of steroids or immunosuppressive therapy in subjects with immune-mediated disease. This may explain the inconclusive results of previous studies in HUS. Novel studies should probably focus on the role of steroids as first-line therapy for immune-mediated forms. Splenectomy, high-dose immunoglobulins or other immunosuppressive drugs have been found to induce remission in some plasma-resistant cases of aHUS, but were ineffective and actually increased morbidity and mortality in others [19]. While there is no rationale for these treatments in genetic forms, it is possible that they may be of some benefit as second-line therapy in patients with anti-CFH antibodies.

Other forms of aHUS
About half of patients with familial and sporadic aHUS do not carry mutations in the above-mentioned genes or anti-CFH antibodies. It is likely that mutations in other known or unknown genes encoding proteins involved in the alternative pathway of complement are associated with those familial cases. A wide variety of triggers for sporadic aHUS has been identified, including various bacteria and viruses, drugs, malignancies, transplantation, pregnancy and other underlying medical conditions (e.g. scleroderma, anti-phospholipid syndrome, lupus; reviewed in [1]). Infection caused by neuroaminidase producing Streptococcus pneumoniae accounts for approximately 30–40% of sporadic aHUS cases [1]. However, in a substantial number of cases with aHUS no clear underlying triggering condition can be identified (idiopathic aHUS). A genetic abnormality in still-unknown genes may underlie these sporadic cases of unknown aetiology.

These forms are beyond the scope of this review and will not be addressed further.

Membranoproliferative glomerulonephritis
Membranoproliferative glomerulonephritis (MPGN) is an uncommon cause of chronic nephritis that occurs principally in children and young adults. At variance with HUS, in MPGN the cell target is the mesangium, as documented by mesangial cell proliferation and increased mesangial matrix deposition [42]. MPGN may be secondary to autoimmune diseases, chronic infections and malignancies, or may be idiopathic. Primary idiopathic MPGN is more rare and accounts for approximately 4% and 7% of primary renal causes of nephrotic syndrome in children and adults, respectively [43]. Half the patients present with nephrotic syndrome, the others with mild proteinuria, 20% with macrohaematuria. About one-third develop hypertension at onset of the disease. Children with MPGN have an unfavourable prognosis and develop ESRD during late childhood or early adolescence. Three distinct types of primary MPGN have been described based on immunofluorescence staining, ultrastructural appearance and complement profiles. The most common variant MPGN type I is characterized by the predominant presence of subendothelial deposits; MPGN type II is characterized by dense deposits in the basement membrane [42]. The term MPGN type III is used to describe the presence of subendothelial and subepithelial deposits [44].

Immunofluorescence glomerular staining for complement C3 and lack of staining for immunoglobulins are common in MPGN type II, but were also reported in MPGN type I. Similarly, markers of continuous complement activation and C3 turnover, such as low C3, low factor B and APH50, are common in MPGN type II but were also observed in MPGN type I [45].

MPGN types I and II and hypocomplementaemia have been reported in a few patients who lack CFH in plasma, due to either homozygous or compound heterozygous mutations that affect protein secretion [46–48], or who have a mutated defective CFH protein that is secreted in plasma [49]. Fifty to 80% of patients with MPGN type II are positive for the nephritic factor (C3NeF), an autoantibody against the alternative pathway convertase C3bBb that stabilizes the complex and makes it more resistant to CFH-mediated decay accelerating activity [50–53]. However C3NeF is also observed in patients with partial lipodystrophy, meningococcal meningitis and even in healthy individuals [54]. Nephritic antibodies that activate either the classic pathway (C4NeF) or the alternative pathway (C3NeF and Nf; the latter binds and stabilizes the C3bBbP C3 convertase) have been found in 30% of patients with MPGN type I and in 60% of patients with MPGN type III [55]. Finally, an anti-CFH mini-autoantibody in the form of a 46 kDa monoclonal immunoglobulin lambda light chain dimer was isolated from the blood of a woman with a mixed form of MPGN types I/II.
with both subendothelial and dense intramembranous deposits [56]. Thus, quite distinct abnormalities, including mutations in CFH, the presence of circulating CFH inhibitory antibody and nephritic factors, result in continuous C3 activation, leading to glomerular damage in these forms of MPGN.

Many individuals with MPGN type II develop drusen, whitish-yellow deposits within the choriocapillaris–Bruch’s membrane–retinal pigment epithelial interface [42,57]. Over time, vision can deteriorate and subretinal neovascular membranes, macular detachment and central serious retinopathy develop in about 10% of patients [58]. These eye lesions and symptoms are similar to those seen in AMD, the most common cause of blindness in the population over 60 years of age [59]. A common CFH polymorphism, causing a tyrosine-to-histidine change (Y402H), has been shown to be a susceptibility factor for AMD in several studies [59–62]. Interestingly, 85% of patients with MPGN type II have the H402 variant [63], which supports a common pathogenetic CFH-related origin of MPGN type II and AMD.

**Therapy**

At present, there is no universally effective treatment for idiopathic MPGN [64–67] (Table 1). MPGN is a rare glomerulonephritis with a protracted natural history, which makes studies on treatment logistically difficult to perform. Most studies are confined to MPGN type I and have a relatively short-term follow-up period. Only a handful of randomized controlled trials have been published with sufficient power to determine the benefits of therapy for MPGN. The use of variable end-points (e.g. reduction in proteinuria, renal function measured using variable techniques) confounds the data further. Several therapeutic regimens have been tried, including the use of corticosteroids and other immunosuppressive drugs, anti-coagulants and anti-platelet agents, non-steroidal anti-inflammatory agents, plasma infusion and plasma exchange [67].

**Immunosuppression**

Different immunosuppressive drugs and regimens have been attempted in patients with MPGN with the objective of halting the abnormal immune response underlying formation of the nephritic complement-activating antibodies, with variable results.

Long-term controlled studies have suggested that children with idiopathic MPGN type I having nephrotic range proteinuria, interstitial disease or renal insufficiency may benefit from corticosteroid therapy. Benefits in children include stabilization of the renal function, slowing of the decline in GFR and a decrease in proteinuria [64,68]. In a study by Braun et al. [69], the long-term (at least 5 years) follow-up of patients with MPGN types I and III treated with a prolonged alternate-day prednisone regimen was reported. In patients with MPGN type I renal survival rates improved, and findings on repeated kidney biopsy at 2 years demonstrated an increase in capillaries with open lumina and a decrease in mesangial matrix and cellularity [69]. However, an increase in glomerular sclerosis and tubular atrophy occurred. In the same study patients with MPGN type III responded poorly to prednisone [69]. On the other hand, in two children with MPGN type I who showed renal disease progression despite prolonged prednisone administration, steroid withdrawal was associated with slowing of renal function deterioration, decrease in proteinuria and elevation of plasma protein and C3 levels [70], suggesting that in steroid-resistant cases withdrawal of therapy may be of some benefit.

Overall, the available data in MPGN types I and III suggest high-dose, alternate-day steroids for a period of 6–12 months (40 mg/m² on alternate days) [71]. If no benefit is seen, discontinuation of steroids with close follow-up and attention to conservative treatment (that is, blood pressure control, use of agents to reduce proteinuria and correction of metabolic abnormalities) is recommended.

In a randomized placebo-controlled study [65], children with MPGN type II had no better response to prednisone than to placebo, with treatment failure, defined as a creatinine greater than 350 mmol/l, in approximately 56% and 60% of patients, respectively. Available data on steroid therapy in adults with MPGN type II suggest a similar lack of efficacy [66].

Anecdotal reports describe benefits of calcineurin inhibitors in steroid-resistant MPGN. In two children with MPGN type I with suboptimal response to prolonged course of steroid, rapid and complete remission of the nephrotic syndrome was achieved after initiation of tacrolimus [72]. In another study in eight children with MPGN types I and II and steroid-refractory nephrotic syndrome, the addition of cyclosporin was associated with remission, defined as proteinuria < 500 mg/day [73]. Recovery from the nephrotic syndrome was reported in another patient with MPGN type II upon cyclosporin administration [74]. However, other long-term follow-up studies in small groups of patients failed to find any improvement in renal survival in MPGN type II patients given calcineurin inhibitors [42]. Anecdotal reports documented some improvement of renal function and reduction of proteinuria in a few patients with steroid-resistant MPGN by administration of mycophenolate mofetil [75].

**Anti-platelet agents**

Anti-platelet and non-steroidal anti-inflammatory agents have been proposed for the treatment of MPGN. Inhibition of platelet activation, mesangial proliferation and alteration of renal haemodynamics are the probable mechanisms that underlie the therapeutic benefits of these drugs. In a prospective clinical trial, dipyridamole and aspirin administered...
over 1 year to patients with MPGN type I reduced the incidence of renal failure at 3–5 years, but the renal survival rate was not different from the untreated group at 10 years [76]. Another study using these two agents in both MPGN types I and II showed significant reduction in proteinuria at 3 years in the treated group [77]. One small uncontrolled study of MPGN type I in children found improved outcome and attenuated inflammation on biopsy with the administration of a combination of prednisolone and dipyridamole [78].

Plasmapheresis and plasma infusion

Plasmapheresis has been attempted in a few patients to remove nephritic factors from blood. In one study, one of three adults with MPGN type II experienced improvement in serum creatinine during plasmapheresis [79]. In two cases with recurrent MPGN type I post-transplant, plasmapheresis with albumin replacement improved renal graft function and light microscopy [80, 81]. Another study reported success using plasmapheresis to treat a 5-year-old boy with recurrent MPGN type II after transplantation [82]. Of note, in the above reports during plasmapheresis, exchange was albumin and not with fresh frozen plasma. A 15-year-old girl with rapidly progressive recurrent MPGN type II in the allograft underwent 73 plasmaphereses that stabilized her creatinine and improved creatinine clearance. During the course of therapy, serum C3NeF decreased and C3NeF activity was detected in the removed plasma. However, because of the morbidity of repeated plasmaphereses, treatment was discontinued and graft failure ensued [83].

Infusion of fresh frozen plasma has been shown to be an effective therapy in those few patients with MPGN type II secondary to CFH mutations. This therapy provides normal CFH, thus correcting the CFH functional defect. In two girls with a homozygous CFH mutation, plasma infusion (10–15 ml/kg/day) was given regularly every 14 days [49]. The treatment interval was derived from measured half-life of CFH of about 6 days. Except for one episode of hypotension in one patient and a few episodes of unspecific abdominal pain in the other, which were interpreted as symptoms of allergic reactions and could be reversed by temporary discontinuation of plasma and administration of an anti-histaminic, the treatment was well tolerated and renal function remained normal in both patients [49]. Similar results were seen in CFH-deficient Norwegian pigs that segregate an I1166R mutation in CFH and develop MPGN type II [84]. These animals died by 7 weeks of age, but developed normally with plasma replacement therapy [84].

Renoprotective therapies

Anti-proteinuric renoprotective therapies, such as angiotensin-converting enzyme inhibitors (ACEI), angiotensin II type-1 receptor blockers (ARBs) and lipid-lowering agents, have been shown to slow renal disease progression in several glomerular diseases [85] and could be theoretically of benefit to also halt progressive renal dysfunction in MPGN.

Anecdotal reports support a beneficial role of angiotensin blockade in patients with MPGN. Long-term therapy with enalapril in a child with MPGN was associated with complete disappearance of proteinuria [86]. However, this therapy was accompanied by a fall in GFR. In another child, remission from heavy proteinuria was achieved by combination of ACE inhibitors and ARBs [87]. In a study in 30 patients with MPGN, hypertension and mild to moderate impairment of renal function, who were randomized to receive diuretics and beta blockers (control), a calcium antagonist (nifedipine) or enalapril over 9 months, treatment with enalapril was associated with decrease in serum creatinine and 24 h albuminuria and increase in creatinine clearance, whereas these parameters did not change or even worsened in the control and nifedipine groups [88].

Transplantation

Despite any treatment, progression to ESRD occurs in about half of patients with MPGN types I or II within 10 years of diagnosis [89].

In around 40–48% of patients with MPGN type I who received a cadaveric kidney allograft, disease recurred on the graft [90]. The incidence is even higher in recipients of an identical living related donor graft [91]. Recurrence has a detrimental effect on graft survival and indeed, graft survival was significantly worse in patients with recurrence than in patients without recurrence, with a median survival of 40 months after diagnosis of recurrence [90].

MPGN type II recurs in the majority of renal allografts and although progression to ESRD is not inevitable, many allografts fail. The North American Pediatric Renal Transplant Cooperative Study database reports the graft outcome of 75 patients with MPGN type II [92]. Five-year graft survival for these patients was significantly worse (50%) compared with the database as a whole (74-3%). The primary cause of graft failure was recurrent disease that occurred in 67% of patients with post-transplant biopsies [92]. Although there was no correlation between pretransplantation presentation, pre- or post-transplant C3 levels and either disease recurrence or graft failure, there was a strong association between heavy proteinuria and disease recurrence.

In another survey on a European cohort of 43 MPGN type II patients who received a kidney graft, MPGN recurred in 49% of patients [93]. Younger age at initial diagnosis and the presence of crescents in the original biopsy were associated independently with recurrence.

There are few data to suggest that any therapeutic intervention have an impact in reversing post-transplant MPGN recurrences, although isolated reports have described that the use of plasmapheresis is of benefit both in MPGN types I and II [81, 91, 94].
Future directions

Clinical research efforts are aimed at identifying more specific approaches that may interfere with the primary cause of the disease in different forms of aHUS and MPGN.

In genetic forms, a main target is to replace the missing or mutated protein. In this regard, efforts are ongoing to obtain a concentrated plasma of CFH for patients with aHUS and MPGN associated with CFH mutations [95]. A plasma fraction that substantially retains the beneficial activity of whole plasma would reduce the total amount of plasma proteins infused, limiting the risk of allergic reactions and fluid overload, while providing the patient with enough active molecule. The active plasma fraction in lyophilized form could be made available to centres that lack facilities for plasma exchange: it would allow better and more prompt treatment of the disease at considerably lower cost, and would limit the risk of viral infection. The problem with CFH replacement therapy is that the concentration of CFH in blood is high and the half-life of the protein is relatively short. A second approach for replacement therapy is gene therapy. Advances in vector safety and transfection efficiency will, hopefully, soon render gene therapy a realistic option for these patients.

Rituximab, an anti-CD20 antibody that has proved efficacy in the treatment of CD20+ lymphoproliferative disorders [96] and some autoimmune diseases [97], could be used as a rescue therapy to treat acute episodes in patients with aHUS and CFH-antibodies who are plasma-resistant. The rationale for using rituximab rests on previous studies in patients with thrombotic thrombocytopenic purpura (TTP), a thrombotic microangiopathy related to aHUS, who had anti-ADAMTS13 autoantibodies. Indeed, rituximab led to clinical remission of acute episodes of TTP refractory to plasma exchange, to reduce the anti-CFH antibody titre and prevent recurrences on the kidney graft, has been tested recently in patients with thrombotic thrombocytopenic purpura (TTP), a thrombotic microangiopathy related to aHUS, who had anti-ADAMTS13 autoantibodies. Indeed, rituximab led to clinical remission of acute episodes of TTP refractory to plasma, and rituximab prophylaxis during remission caused antibody disappearance and prevented relapses [98,99]. The effect of prophylaxis with rituximab combined with plasma exchange, to reduce the anti-CFH antibody titre and prevent recurrences on the kidney graft, has been tested recently in a patient with chronically elevated levels of anti-CFH antibodies who developed ESRD despite intensive plasma treatment [100]. The post-transplant course was uneventful and at 46 months follow-up the graft function was good and at 46 months follow-up the graft function was good and at 46 months follow-up the graft function was good and at 46 months follow-up the graft function was good and at 46 months follow-up the graft function was good and at 46 months follow-up the graft function was good and at 46 months follow-up the graft function was good and at 46 months follow-up the graft function was good.

A second approach for replacement therapy is gene therapy. Advances in vector safety and transfection efficiency will, hopefully, soon render gene therapy a realistic option for these patients. Eculizumab, a humanized antibody against C5 that blocks the formation of the terminal complement complex, has been shown to reduce haemolysis, haemoglobinuria and the need of transfusions in patients with paroxysmal nocturnal haemoglobinuria. In CFH-deficient mice, which develop spontaneously MPGN type II dependent on C3 activation, treatment with a monoclonal antibody to mouse C5 ameliorated the course of renal disease [101]. The latter data support the potential use of a C5 inhibitor in patients with MPGN type II.

Another complement-blocking approach under investigation is based on the use of membrane-targeted analogues of complement receptor 1 (CR1) [102]. Once available to the market, such compounds could be useful to protect the kidney locally from complement attachment in both aHUS and MPGN.

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